

What to Say About Scientific Evidence

NOT all the letters we receive each week convey hard scientific facts ripe for publication. A few chide us for omissions and commissions and some question our ability to publish a hymn sheet, let alone a learned journal. But one received recently set off an interesting train of thought. The writer had singular views on low temperature physics, and was outraged that the journal in the past had declined his contributions on this subject. "Mr Brimble, one of your predecessors," he wrote, "refused to see the Truth of this proposition; he died shortly thereafter". The implication was palpably *post hoc ergo propter hoc* and we tempt providence by turning our backs again on this Truth. The question that seemed fascinating was; granted that a scientist could see flaws in the implication of this indelicately worded letter, how well could he see flaws in the logic of his own work when it had all the trappings of a scientific paper?

The most dangerous pitfall in scientific reasoning is buried little deeper than the *post hoc* proposition. It is the acceptance of the statement 'if A implies B, then B implies A'. To take an example of such reasoning which does not these days point an accusing finger at anyone, 'if the Earth being at the centre of the Universe with the Sun rotating daily around it implies a succession of night and day, then our observation of night and day implies an Earth-centred Universe'.

Mathematicians are particularly aware of the snags in this because the issue is that of sufficient and necessary conditions. The truth of B is, of course, a necessary but not sufficient condition for the truth of A.

In observational science the chances are relatively slight that we can measure enough of the implications of A to rule out any other hypothesis than A. A common problem is of failure to identify enough alternative hypotheses. A coin is tossed 1,000 times—it shows 'heads' in 600 cases. It would be totally wrong, for instance, to say that 600 'heads' makes it highly credible that the coin is weighted. Instead, it may be bent, the experimental procedures may be at fault, or the experimenter may even be lying; all of these alternative hypotheses have to be assessed carefully. It is the mark of the successful scientist that he has rich enough an imagination to look for these alternative hypotheses, particularly when the conventional one is popular.

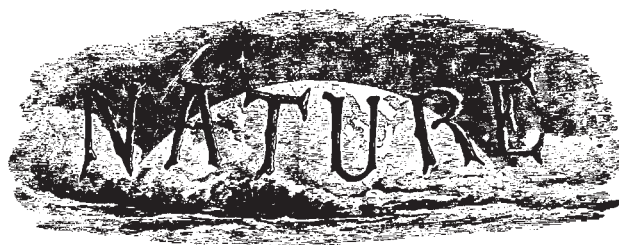
An unseemly phenomenon familiar to all scientists is the rolling bandwagon, on which our logical fallacy frequently rides. A first-rate new idea is introduced into a subject. It is instantly attractive since it explains a variety of previously mystifying observations. Whole laboratories swing into action and within months, papers are emerging which run thus—the new idea implies the following predictable observations; we have made these observations and therefore we have helped verify the idea. A frequent phrase is "we consider that our observations strongly support the notion that . . ." This may be pure nonsense, and history takes its toll of such papers. Even if the observations are indeed concordant with the hypothesis and hindsight shows them to have been of use in

establishing the validity of the hypothesis, they cannot support the new idea unless they clearly also refute all other hypotheses or at least render them all highly incredible. Such crucial experiments are rarely possible. When the experiment is not a crucial one, the most that the experimenter should say is that he is bringing forward new evidence to bear on the issue. The validity of new concepts in science is usually established ultimately by processes not dissimilar to those involved in the determination of guilt or innocence in a court of law, and if the witnesses each bring their own opinion as well as their evidence this rapidly discredits both witness and evidence.

Moreover, the desire to vote fashionably can seriously inhibit science by requiring any imaginative thinker, with an alternative hypothesis which he wishes to test, to sift out evidence and decide how much of the published data has not been tarnished. Preconceptions not only creep into 'interpretation' but often even control the way in which the experiment is performed. Scientists are sometimes guilty not only of accepting, suitably camouflaged, the proposition that B implies A, but are also capable of designing experiments that will only yield B as an answer, or designing a rationalisation to remove all not-B from their data.

The solution to the problem of evaluating scientific evidence lies with the practitioner himself. There is no point in attaching to research departments experts in scientific method alone. Scientists must indulge in more healthy scepticism of their own and other people's treatment of data; there is a continuing need of strong and informed criticism of the way science handles its evidence. When properly done this is the greatest benefit of the refereeing system.

100 Years Ago



Dynamometers, by R. S. Ball, LL.D., F.R.S.

If we adopt that force which acting on one gramme for one second will impart the velocity of one centimetre per second as the unit, then one million of such units is a convenient magnitude for practical purposes. The large figures on the dynamometers represent these million units, for which it is hoped that ere long a suitable name will be adopted. The dynamometers are intended for educational purposes. They are exhibited to the Association with the desire of aiding the present movement in favour of an improved system of fundamental unity.

A report from the British Association

From *Nature*, 8, 497, October 9, 1873.



OLD WORLD

European Science Foundation Formed

EVEN the most confirmed sceptics must be impressed by the speed with which the academies and research funding bodies of Europe have set up a European Science Foundation. At a two day meeting in Paris last week fifty western European academies and research councils* decided in principle to set up a foundation following less than a year of detailed consideration.

The foundation's aims are to promote

- cooperation in fundamental research
- the free movement of research workers
- the exchange of ideas and knowledge, and
- the harmonisation of European research programmes.

A preparatory commission which will produce a constitution for the foundation has been created and it is hoped to sign a charter forming the foundation in May next year, setting up its headquarters at a place to be decided.

Research councils across Europe keep in touch with each other—the Medical Research Councils for example meet regularly—and this cooperative approach to problems even spread to the Science Research Councils which had a meeting at Aarhus in 1972. Many learned societies and academies have also set up exchange arrangements in the past decade.

But the real stimulus for the formation of the science foundation came from Signor Altiero Spinelli of the European Commission. He galvanised the European research councils and academies into action by the simple expedient of publishing, in the summer of 1972, his proposals for a common scientific, technological and industrial policy under the title *Objectives and Instruments of a Common Policy for Scientific Research and Technological Development*. One key proposal was that a European Science Foundation should be formed. But the proposal stated that it should include development work within its remit.

The reaction of Europe's scientific bodies was predictable. At a meeting held at the Royal Society in December last year the fifty academies of Europe discussed the proposal and decided that they did not want a Brussels bureaucracy trying to run science, they did not want a foundation for science that included

the politically sensitive development side, they did not want a foundation limited merely to the community's nine, and they did not know whether they even wanted a foundation of any sort.

But the meeting—hostile though it was to Spinelli's proposals—was not entirely negative. The academies and research councils found themselves in almost complete agreement and decided to examine the possibilities of setting up a foundation themselves. A second meeting was held in Munich in April.

By then the situation had changed. Britain, Denmark and Ireland had joined the European Community, the commissioners had changed their jobs, Spinelli's original proposals, opposed by Britain and France, were due shortly to be quashed, and Dr Rolf Dahrendorf was now the commissioner responsible for European science. He attended the Munich meeting.

His response to the attitude of the research councils and academies was

encouraging. The commission, he explained, was now willing to see a foundation devoted purely to fundamental science. Furthermore the commission was willing for countries outside the nine to be included and it was happy to have the scientists themselves organise the body.

By the end of the Munich meeting the balance had swung from the academies and research councils being largely opposed to a foundation, to their being cautiously in favour.

In the months between Munich and last week's Paris meeting the questions which had to be answered included defining the relationship between the foundation and existing bodies, defining its relationship with the European Community and countries outside the EEC, and deciding which bodies should belong to the new foundation.

These questions have now been answered. The European Commission, subject to the approval of the council

SOVIET SCIENCE

Quick Clay Tube

THE most spectacular achievement of the reign of Peter the Great was the building of his new capital in the Neva marshlands. The lack of a firm subsoil, however, is a considerable problem to the civil engineering planners of modern Leningrad—but now one major difficulty at least has been met with a solution in the grand tradition of the city's founder.

In driving the tunnels for the new Metro, the "Lenmetrostoi" Trust (specially established for the task) encountered the apparently impassable obstacle of a 450-metre long bed of quick clay—the legacy of a pre-glacial tributary of the Neva. The situation was not suitable for treatment by the standard method—electrolyte injection—and accordingly a more radical treatment was proposed, namely, the freezing of the bed to a temperature of -10°C .

Civil engineering operations on quick clay regions are notoriously dangerous—the Surte disaster, which carried a railway a main road and three hundred homes into the River Gota was initiated by a pile driver employed on the foundations of a new building. The "Lenmetrostoi" teams, however, are safely operating a mechanical pick, with a nominal progress rate of 33 cm per shift, through the former quick clay bed that they have transformed into an "iceberg". They

are even able to use explosive charges to deal with the occasional two-ton granite boulders found in the bed.

How the quick clay was frozen in the first place has not been revealed. The press (*Pravda*, September 26, 1973) only describes the work in progress through the pre-frozen mass, with indium alloy pipes penetrating the work-face, carrying in the refrigerant (calcium chloride solution). It would appear, however that the actual thickness frozen is small—since cracks in the frozen walls of the bore must be closed at once by metal shutters to prevent flooding of the workings by the quick clay beyond the "iceberg".

It is not clear what preventive measures will be employed when the Metro is operating. It is proposed, however, that through the quick clay region the train tunnels should lie not parallel but one on top of the other. Even so, further precautions will surely be necessary if the thixotropic properties of the bed are not to be activated by the vibration of the trains. The cost of constructing this section of the Metro has already risen above 2 million roubles (£2,000,000) and (a serious matter in Soviet planning) the construction time has already doubled. It would appear that if the Metro is to operate without causing risk to the city above, the "Lenmetrostoi" engineers will have to find a further "heroic" solution to deal permanently with this quick clay bed.

* The academies and research funding bodies represented were from Austria, Belgium, Denmark, Spain, France, Greece, Norway, Holland, Portugal, West Germany, Ireland, United Kingdom, Sweden, Switzerland, Yugoslavia, Italy.

of ministers, will belong to the foundation, will contribute financially, and will use the foundation as an advisory body on fundamental science. The foundation will include all Western European academies, the qualification for membership being that they finance research. Learned societies without grant-giving powers will not be eligible.

But what will the new foundation do? Initially it will be a talking shop. Sir Kingsley Dunham, Foreign Secretary of the Royal Society, says that "in spite of the plethora of European organisations already in existence, there is still a need for a forum in which to exchange information on what is going on, on both a large and a small scale". A small secretariat will be set up, and the immediate objective will be to sort out areas of science where more cooperation or a change in emphasis on research is needed. It is likely that the foundation's initial budget, contributed by its members, will be about £1 million, some of which will be used to support needy areas—the Flora Europaea programme is one possibility. In time it is likely that the foundation's budget will expand to allow more research to be supported.

Other work could include allocating spare time on accelerators, reactors and telescopes, for example, and laying the groundwork for major cooperative projects such as CERN. It is not intended, however, that the foundation should ever finance such expensive programmes. Multilateral agreements for the exchange of research scientists could be devised. International cooperation in areas where setting up separate organisations would be wasteful can be encouraged—for example in geodesy and geophysics—and such projects as the provision of experimental material for zoologists can be undertaken.

But where the foundation can play its most important role is in providing an overall direction to the hundreds of millions of pounds that the research councils and academies of Western Europe spend between them in sixteen countries.

European governments will have to approve the plan to contribute research council money to a foundation, the Council of Ministers will have to accept the Commission's attitude and the foundation will have to be seen to be doing things early in its existence if it is to be credible. But, as Sir Kingsley Dunham is quick to point out, it will start with an enormous amount of goodwill as it is the creation of the research councils and academies themselves. It is not an organisation imposed from above by governments.

It has been a week of relief and congratulation. Sir Brian Flowers, Chairman of the Science Research Council until this week and a longtime protagonist of the foundation, described the

outcome as very satisfactory, and the enthusiasm governments have evinced towards the foundation—for example Mrs Margaret Thatcher's recent speech (see *Nature*, **244**, 248; 1973) to the Parliamentary and Scientific Committee—suggest that there should be few stumbling blocks there.

The constitution should be prepared by May for signature in Stockholm and the foundation could be underway by late 1974 or 1975; a move that would be wise in view of the impending departure of Dr Rolf Dahrendorf from Brussels. Another Commissioner may be less keen to see the research councils running their own show away from Brussels.

GEOPHYSICS

Zurich Jamboree

THE first meeting of the newly formed European Geophysical Society was held in Zurich last week. The 500 European scientists present, together with a sprinkling of representatives from the United States, assured the success of the meeting. But young graduate students, from all countries including Switzerland, were conspicuous by their absence.

The concept of a European-wide society to cater for the interests of geophysicists originated at the joint meeting of the International Association of Geomagnetism and Aeronomy and the International Association of Seismology and Physics of the Earth's Interior held in Madrid four years ago. The idea was taken a stage further at a meeting held at the University of Reading in 1971 when a committee was set up, with Mr C. R. Argent of the Royal Society as secretary. It has acted as midwife to the society since then.

Professor Keith Runcorn, of the University of Newcastle upon Tyne, said this week that one of the aims of the society is to ensure that European graduate students get the opportunity to present the results of their research to a highly critical international audience. The students will also get the opportunity to hear and meet international experts in all aspects of geophysics. Professor Runcorn, who has been for many years in the forefront of efforts to get the society established, also said that the society should ensure that Europeans will become aware of the possibilities for cooperation with laboratories in other countries. International collaboration is essential to obtain an appreciation of many geophysical problems such as the geology and geodynamics of Europe, said Professor Runcorn.

Emphasis was placed at last week's meeting on interdisciplinary symposia and on topics of special interest to European geologists and geophysicists.

There were sessions on the deep structure of Europe, on which there is new seismic information, and on pelagic sediments, data on which have been recently obtained from drillings in the Mediterranean. The meeting was not only a local affair and the Moon and planets received much attention, with the Mariner 9 photographs of Mars being specially appreciated.

It is no secret that the European Geophysical Society is modelled very much on the highly successful American Geophysical Union and the "frontiers of science" lectures at last week's meeting highlighted the similarity between the two organisations. At Zurich, Professor J. Geiss of the University of Bern, gave a talk on the solar wind and its application to astrophysics and geophysics, while Dr D. McKenzie of the University of Cambridge revealed to his audience the secrets of mantle convection and the Earth's thermal history.

Professor Runcorn wants to see the society provide opportunities for differ-

Televised Science

If science is to become a part of the background to daily life, rather than being enshrined as some taboo topic which is only understood by coldly efficient white coated experts, James Burke and Dr Who, then the first requirement is that scientific topics should be represented in the media. In Britain today, this means that science must be an integral part of television features.

BBC TV has received criticism in the past for its handling of science features, but at least the features group do produce science programmes, and these are reputed to be the best such programmes produced by any national TV network in the world.

The BBC TV features group have recently announced plans for the coming season, and these include "one off" specials such as *The Life Game* (a programme about "evolution in action"), a series on the lives and work of famous scientists and regular features such as *Horizon*, which will be looking at subjects ranging from pygmies to black holes. In addition, old favourites like *Tomorrow's World* will be on British TV screens each week throughout the next few months. *Nature* will be keeping a close watch on these developments—and on the presentation of science through other media including radio and films—and from time to time will be reporting on the success or failure of the media in presenting science to the public.

ent disciplines to appreciate each other's difficulties. In this way many problems will be seen to be common. For example, says Professor Runcorn, those concerned with convection in the mantle will undoubtedly benefit by dialogue with those working on geophysical fluid dynamics of atmospheres and oceans.

NUTRITION

Equation Doesn't Balance

from a Correspondent

DOES the man/food equation balance? Not for long, was the verdict of a two-day symposium held at the Royal Institution, London, recently. The first day was devoted to the problems of feeding an increasing population within the constraints of conventional food production; the current supply of fuel and preservation of the environment.

Averaged over the globe it is easy to be complacent about the rate at which food production is keeping pace with population growth, but several speakers pointed out that such averages serve to mask local inequalities of distribution that are indisputably linked with purchasing power. Professor G. Borgstrom (Michigan State University) explained that with some exceptions most of the major gains, in themselves quite dramatic advances in agriculture, have benefited the satisfied world while the hungry world is growing relatively more hungry. Furthermore, although the growth rate of fisheries has been keeping up with population, this increase has been associated with a lowering of quality, measured by the proportion of the catch converted to animal feeds, where there is an eleven-fold reduction in protein yield (Dr S. J. Holt, International Fisheries Research, Malta). Even this growth of the fisheries is bound to slow down as stocks are overfished—witness the collapse of the anchoveta industry in Peru.

The chairman, Dr A. Bourne (Lancaster University), argued cogently that even now insufficient attention is being paid to environmental balance and that as a result of intensive agriculture, deforestation, urbanisation and pollution, man is being driven into an environmental cul de sac with not enough room to turn. Much of the energy provided by the Aswan dam is used for the manufacture of fertilisers to supplement the fertility of the Nile delta, which it destroyed. Deforestation in China has long resulted in excessive loss of sediment to the ocean. Man is the ultimate predator and even the ocean food he preys on is becoming increasingly polluted by his frenetic and self-imposed industrialisation. Modern intensive agriculture has interfered with the life cycles of nitrogen-fixing organisms and our only means of replacement involves

enormous quantities of energy from fossil fuels. The dangers of emptying the natural reservoirs of indigenous genetic variability are already becoming apparent when we see how narrow is the genetic basis that plant breeders will be able to tap in the future if these centres of variability are destroyed (Dr J. T. Williams, Birmingham University).

On a more personal level, the problems of energy and malnutrition were examined by Professor P. V. Sukhatme (Gokhale Institute, Poona) and Dr E. Wheeler (London School of Hygiene and Tropical Medicine). Protein malnutrition is endemic in India, not because the protein in itself is insufficient, but because the calorie intake is too low to metabolise it. In Bihar, for example, 9% of the population had too little protein in their diet, but 59% had insufficient calories. Equalising the energy purchasing power is the answer to malnutrition here.

It now seems well established that vascular disease and constipation are the price we pay for Western civilisation and the final session of the symposium was devoted to the part played by our degenerate diet. Dr M. Crawford (Nuffield Institute of Comparative Medicine, London Zoo) laid particular stress on the importance of lipids in the diet, especially linoleic and linolenic

acids. In maternal nutrition, these fatty acids may be crucial for the developing brain and nervous system of the foetus. Dr B. Feingold (Kaiser Medical Centre, San Francisco) described some of his successful treatments of children suffering from hyperkinesia and learning difficulty with salicylate free diets, hypothesising food additives as causative agents of this disease.

Finally, Professor D. P. Burkitt (Medical Research Council) and Dr N. Painter (Manor House Hospital) presented evidence for the importance of fibre in the diet. Non-infective bowel diseases, a scourge of the Western world are rarely found in developing countries. Burkitt developed a convincing argument that these diseases are associated with increased pressure in the intestinal lumen and faecal arrest. Cereal fibre, high in hemicelluloses, is lacking and Dr Painter provided the clinical evidence for putting fibre back into the diet. By giving bran, he has had remarkable success in treating patients for diverticular disease.

The drug and food industries and the medical profession might heed Burkitt's analogy with the overflowing sink; too much effort and money is spent on the mopping up operation and not enough on turning the tap off. Synergy, not dominance, is the keynote to survival.

POLLUTION RESEARCH

Modest Increase

OVER £1.2 million was spent by the research councils on pollution research in 1971–72. This is £181,000 more than was spent the previous year. These figures are revealed in a report, *Pollution Research and the Research Councils*, published last week by the five research councils.

The collaboration of the research councils in pollution research resulted from an enquiry in 1969 by the Council for Scientific Policy into the problems of pollution. As a result a working party was set up with representatives from all five research councils first to collate and

examine the relationships of the pollution research programmes of the research councils and, second, to identify opportunities for further liaison and joint action by the councils in certain aspects of pollution research. Since then the working party has incorporated members from outside the councils and it now includes members from industry, University and government departments. The committee now has it in its remit "to advise the government and government sponsored bodies, if requested, on scientific matters relating to the problems of pollution".

Details of where the money goes is shown in the table with the distribution in 1970–71 shown for comparison.

Table 1. Direct Pollution Research Expenditure

Kind of investigation	ARC	MRC	NERC	SRC	SSRC	Total 1971–72	Total 1970–71
Survey, monitoring and dispersal of pollutants	180	2	132	31	—	345	454
Effects of pollutants on man, animals and plants	73	245	236	33	2	589	327
Abatement, reclamation, biodegradation	169	8	5	22	—	204	199
Technological aspects	10	12	5	9	—	36	43
Information, data collection, data handling, data analysis	—	—	8	11	11	30	—
Total 1971–72	432	267	386	106	13	1,204	—
Total 1970–71	198	509	221	74	21	—	1,023

Totals in thousands of pounds sterling.

NEW WORLD

Nerve Gas—The Army's Latest Weapon

by our Washington Correspondent

ALTHOUGH President Nixon announced, in November, 1969, that the United States would never be the first to use chemical weapons in a war, the Department of Defense has carried on an extensive research and development programme on nerve gases and other chemical agents. The latest fruit of the Army's endeavours, so-called binary nerve gas weapons, will be rolling off the production lines in about four years if Congress approves and—perhaps more important—if the Army gets its way with the rest of the armed services and the Administration. In any case, the Army has already begun to mobilise its public relations forces.

As usual in such matters, a pall of secrecy surrounds the details of binary weapons. But their chief feature is that they are made from two relatively harmless chemicals which form a lethal nerve agent only when they are mixed together. The idea is that the two components would be stored separately and they would be loaded into a shell on the battlefield. As the shell is fired, a diaphragm separating the components ruptures and the nerve agent is produced while the projectile is on its way.

The Army is enthusiastic about the possible addition to its chemical arsenal because binary weapons open up the possibility of getting rid of the stockpiles of lethal nerve gases that are now stored in army depots throughout the United States. These generate public alarm and opposition to the entire chemical weapons programme.

But the enthusiasm is not shared by everybody, for there is concern in some circles about the effects of a large new United States chemical weapons programme on international attempts to ban chemical and biological warfare agents. In particular, there is some alarm about the possibility that the development of binary weapons in particular will increase the chances of proliferation of nerve agents not only to countries that do not already possess them but even to terrorist groups.

Congress has been aware of the Pentagon's binary weapons programme for at least four years through testimony given to armed services and appropriations committees. But the hearings have been held behind closed doors and the "sanitised" transcripts of the proceedings have contained virtually no discussion of the possible impli-

cations of the programme. Last month, however, a few members of Congress received a note from the Secretary of the Army which gave a few details of the planned production of binary weapons. This was the Army's first pitch to get its programme accepted.

Among other things, the note indicates that the Army is hoping for nothing less than the total replacement of existing stocks of nerve gas by binaries—a programme several orders of magnitude greater than many observers were expecting. To give some indication of the scope of the programme, a report published by the Stockholm International Peace Research Institute (*The Problem of Chemical and Biological Weapons*, II, 1973) estimates that there are between 15,000 and 20,000 tons of nerve gases now stockpiled in the United States.

Specifically, the Army's note says that the Pine Bluff Arsenal—a chemical weapons facility in Arkansas—has been selected to produce one component of a binary munition. The component will be "similar to chemicals used by the pesticide industry [whose] characteristics closely resemble those of an insecticide for home use", and it will be placed in a special binary shell, also to be manufactured at Pine Bluff. The second component, which will be made by industry, will be loaded into the shell on the battleground.

What the note does not say is when the munition will be produced, how much it will cost, what type of nerve gas will be generated, or whether open air testing will be carried out. In short, apart from the designation of Pine Bluff, the notes says little that is not already known.

According to an Army spokesman, however, production of binary weapons is set for 1977 if Congress comes up with sufficient money. If past records are anything to go by, that should not be much of a problem, for Congress has so far given the Army everything it has asked for to support the programme. In the past three years, for example, research and development on binary weapons has cost \$12.4 million, increasing from \$2.9 million in 1971 to \$5.4 million last year. The nerve agent produced in the binary will, again according to the spokesman, be non-persistent, and another Pentagon official confirmed that it would be very similar to GB, a nerve gas developed in Germany during the Second World

War (but never used) and now heavily stockpiled in the United States. GB is lethal when inhaled or absorbed through the skin.

As far as testing is concerned, it should be remembered that the Army suffered an embarrassing setback to its nerve gas testing programme when, in 1968, a faulty tank on an aircraft sent a cloud of nerve agent outside the testing area in Dugway, Utah, and killed several thousand sheep. That incident led Congress to pass a bill requiring the Secretary of Defense to give at least 30 days' notice of impending tests of lethal agents. Some observers of the binary programme have thus been waiting for such a notice as a signal that procurement of binary weapons is imminent, particularly since General William Gribble, Chief of the Army's Research and Development programmes, told the House Armed Services Committee in 1972 that "open-air testing with lethal agents will be requested to confirm weapon efficiency of the binary 155 mm projectile prior to procurement".

The Army is attempting, however, to by-pass the testing stage. Mr Tom Dashiell, a Pentagon official concerned with the binary programme, said last week that there are no plans to conduct open-air tests with lethal agents. Considerable testing has taken place with non-toxic binary simulants, he said, and such testing has already proved the reliability of the binary concept. It is also believed that the Army conducted at least one test with lethal binary weapons before Congress passed the 1969 restrictions.

As for the military implications of the production of binary nerve gas, it is perhaps worth noting that in references to the weapons during Congressional testimony so far, its military effectiveness has been scarcely mentioned. Yet, binaries would be less effective than conventional nerve gas weapons because hydrogen chloride would be produced as a by-product in the binary reaction. Thus, not only would the nerve gas payload per weapon be reduced by about 30%, but the gas would also no longer be odourless. Moreover, since time would have to be given for the binary components to react, the weapons could not be used at short range or at low altitudes.

The Army is thus gearing up to sell Congress on the idea of binary weapons, and its public relations is likely to

emphasise the safety features of the munitions, compared with conventional nerve gas weapons. An indication of the likely campaign comes in the note to Congress which said that "the binary munition offers a major advance in safety over current chemical munitions . . . their development is intended to obviate the hazards normally associated with the manufacture, transportation, storage, and disposal of the current family of lethal chemical munitions. An Army spokesman added last week that binaries "represent a quantum jump in safety".

The timing of the note to Congress is also worth noting. This week (on October 3 and 4), the House Armed Services Committee held two days of hearings on the storage and transportation of nerve gases. The reason for the hearings was essentially a public outcry that has arisen over the storage and possible relocation of nerve gas weapons at the Rocky Mountain Arsenal on the outskirts of Denver. The arsenal holds obsolete stocks of mustard gas, phosgene and GB in M-34 cluster bombs, which the Army has promised to destroy, together with a quantity of GB which forms part of the deterrent stockpile. Since the arsenal happens to be near to the North-South runway at Stapleton International Airport, Denver residents are understandably unhappy and want the stuff removed. Then, when word leaked out that the Army was considering shipping some of the nerve gas to Tooele Arsenal in Utah, an even louder outcry went up. The Army has the problem under study again, and its decision is likely to be announced at the hearings. It will not let a chance like that go by, however, for doing a little proselytising for its new, safe weapons.

So far, since there has been little public discussion of binary weapons, there has also been little public opposition to them. When it comes, however, it is likely to take two chief tacks. The first is whether or not the expense of making the stockpiles safer is justified. And the second is the effect of binary weapons on international agreements to limit the production and spread of chemical and biological weapons. The second argument is undoubtedly the more important.

As for the economic aspect, Dr Matthew Meselson, Professor of Biochemistry at Harvard, estimated last week that the total cost of developing binary weapons and detoxifying existing stocks of nerve gas could be as much as \$500 million. He pointed out that so far, in spite of widespread public alarm, the Army has a good safety record with its nerve gas stocks, and he suggested that the money could be better spent elsewhere. The Army is

likely to argue, however, that the development of binaries will actually save money because there would no longer be large costs associated with the maintenance of stockpiles of highly corrosive nerve agents. It is estimated, for example, that weapons packed with conventional nerve gases have a shelf life of only about 10 or 15 years. But Meselson is sceptical of that argument, pointing out that the weapons that have given trouble—some M-55 rockets and M-34 cluster bombs—have been either destroyed or are about to be detoxified, and maintenance costs of the stockpiles will shrink in any case.

The international implications are more difficult to predict. Although the United States has never ratified the Geneva Protocol of 1925, outlawing the use in war of chemical and biological weapons (see box), President Nixon's 1969 announcement that the US will relinquish first use of chemical weapons and abandon biological weapons entirely—including their production, storage and use—at least signifies that the United States is interested in inter-

national CBW control. The development of a new generation of nerve gas weapons could, however, damage that impression and make the UN Chemical Warfare disarmament talks, which have just completed their fifth fruitless session in Geneva, even more difficult.

Of great concern to some observers, is the effect that binary production could have on proliferation. Nerve gas weapons are costly to produce, chiefly because of the difficulty of building a plant to deal safely with the extremely toxic and corrosive chemicals. Production of the binary components for nerve agents does not, however, carry such a penalty—a country with an insecticide industry and some leaked American technology would probably be able to produce at least a binary G-agent, according to Julian Perry Robinson, chief author of the SIPRI study (see *New Scientist*, 58, 4; 1973). One step further, the development of binary weapons may even open up the frightening possibility that nerve agents would be within the reach of terrorist organisations.

Hope for the Protocol

by our Washington Correspondent

ALTHOUGH the United States Army is pushing ahead with plans to develop a new generation of lethal nerve gas weapons (see accompanying article), some observers of the United States' chemical and biological warfare posture believe that the time may now be ripe for the government to ratify the 1925 Geneva Protocol on chemical and biological warfare. The protocol, which was negotiated after the extensive use of poison gas during the First World War, outlaws the use of chemical and biological weapons in war. But the United States has never ratified it.

When it was first submitted to the Senate for approval in 1926 (all such treaties entered into by the US must be approved by a two-thirds vote of the Senate), the protocol ran into opposition from the chemical industry and the American Chemical Society—which has since reversed its stand—and it was never acted upon. In 1969, however, President Nixon made his historic announcement that the United States would renounce the first use of chemical weapons in war and abandon biological weapons completely; the following year, he again sent the Geneva Protocol to the Senate for ratification. But it then fell foul of the Vietnam war.

Largely because the United States forces in Vietnam were using herbi-

cides and CS, the Administration insisted that such agents are not covered by the protocol. (The British government has taken a similar position over CS.) But the Senate Foreign Relations Committee, under the chairmanship of Senator J. William Fulbright, maintained that such agents do fall within the scope of the protocol—a viewpoint which was affirmed by the UN General Assembly in 1969 by 80 votes to 3—and refused to act on it until the Administration altered its position. Until the Geneva Protocol is ratified, however, the United States will not ratify a treaty on biological weapons which was signed last year.

Three factors were suggested last week by sources on Capitol Hill and in the Arms Control and Disarmament Agency which may lead to a compromise between the Administration and the Senate on the matter of herbicides and tear gases, however. The first is the ending of the Vietnam war, which no longer puts the US in the embarrassing position of supporting CBW control at the same time as it is using chemical agents in war. The second is two internal reports prepared for the Department of Defense which indicate that the agents are of only marginal value in any case. And the third is the change of leadership in the State Department. As one Congressional source put it, with Kissinger and Fulbright lunching together every other day, anything can happen.

NEWS AND VIEWS

Calcium Triggers Release of Packaged Secretions

THE mechanism by which neurotransmitters, hormones, histamine and other packaged secretions are released from cells is of great pharmacological importance. The release follows a specific stimulus, usually depolarisation or attachment of a chemical agonist to the plasma membrane. Evidence has accumulated that in many different systems coupling of stimulation and secretion requires the presence of calcium ions in the extracellular medium; Ba^{2+} or Sr^{2+} but not Mg^{2+} can substitute for Ca^{2+} . In certain situations, however, this requirement has not been demonstrable, and the role of Ca^{2+} in stimulation-secretion coupling has been doubted by some investigators. The possibility nevertheless remained that Ca^{2+} might be released from an intracellular store analogous to the sarcoplasmic reticulum of muscle. Such smooth membrane Ca^{2+} storage vesicles have a long evolutionary history, since they are present in slime moulds, and they may be widespread in animal cells. To resolve this problem, ways of measuring and controlling the concentrations of Ca^{2+} in the general cytoplasm, as opposed to vesicles, are required.

During the past few years, progress has been made in estimating cytoplasmic Ca^{2+} concentrations with the luminescent protein aequorin. These investigations have confirmed that there is an increase in Ca^{2+} in the cytoplasm after membrane depolarisation and preceding muscle contraction, and also in stimulated presynaptic nerve endings which are releasing neurotransmitters. Recently a calcium ionophore, A23187, which increases the permeability of membranes to Ca^{2+} , has become available; in the presence of the ionophore the concentration of Ca^{2+} in the cytoplasm can be varied by altering the concentration in the extracellular medium. Foreman and his colleagues (see page 249 of this issue of *Nature*) have made use of

the ionophore to analyse the role of Ca^{2+} in triggering the release of histamine from rat peritoneal mast cells. When peritoneal cells were exposed to A23187, $^{45}\text{Ca}^{2+}$ uptake was increased and histamine was released in proportion to the amount of Ca^{2+} in the extracellular medium in the same concentration range as required for release by antibody and antigen. In the presence of A23187, Sr^{2+} in the medium also released histamine.

These results suggest that an increase in cytoplasmic calcium concentration is the normal trigger for release of histamine and other pharmacologically active agents from mast cells. The subsequent events are still speculative. The interesting parallelism between the ions bringing about release of packaged secretions and those triggering the contraction of muscle has led to speculations that an actomyosin-contractile system in the peripheral cytoplasm participates in granule release. The dependence of the release on metabolic energy and its inhibition by cytochalasin are consistent with this possibility. Ca^{2+} is known to interfere with assembly of tubulin into microtubules, so it seems unlikely that assembly of microtubules is required for secretion. Coordinated action of microfilaments providing a motile force and microtubules providing guidance could, however, occur.

Since the concentration of Ca^{2+} in the cytoplasm may be an important regulator, not only of the actomyosin system, but also of other reactions such as the assembly and breakdown of microtubules in cell division and activation of certain enzymes, the discovery of an ionophore by which the concentration of calcium in the cytoplasm can be varied is likely to have many applications in cell biology.

From a Correspondent

When did Plates Start Breaking Up?

ONE of the central pillars of geology is the doctrine of uniformitarianism, which goes back to the 1830s and Lyell. The concept that geological processes have been of much the same intensity throughout the whole of geological time is very simple. There is wide consent on uniformitarianism and whatever controversy it raised in the nineteenth century, when catastrophism was its rival, it is taken for granted now. Which makes it fascinating when geologists suddenly find that they have been using it unthinkingly and perhaps mistakenly.

When plate tectonics was first proposed, it was clear that in some parts of the Earth continental plates are colliding with each other. Such a process is going on at present along a complicated belt of orogeny or mountain building from the Alps to the Himalayas. Since today separate continental fragments can be seen being welded together, the natural reaction is to suppose that similar plate tectonics concepts have always applied and that continents as they are at present have been put together by a steady process of mosaic building. Of course, the

process is not entirely one of building because there are break-up processes—rift formation—going on simultaneously. The question is whether these activities have been going on since the beginning of geological time and obviously the complexity of unravelling the past gets greater the further back one tries to go.

Piper, Briden and Lomax in this issue of *Nature* (page 244) find a neat way to answer the question without having to do any of the unravelling. The Precambrian area of Africa has a pattern of stable blocks between which are orogenic belts. These belts, on a plate tectonic interpretation, are ancient convergent zones—"Precambrian Himalayas". There are, however, some geological reasons to doubt this explanation and to suggest that the belts are ensialic—that is, that they have their origins in continental rocks.

Piper *et al.* test these hypotheses by using palaeomagnetic data. They construct polar wander paths for the blocks separately and show that in the Precambrian the poles for the separate blocks follow rather similar

paths. The same is true when South America is included in the analysis. The inference is quite strongly that Africa and South America behaved as a unit for the whole of the Precambrian, and indeed the authors bring forward some rather less compelling evidence that North America belonged to this unit until about 1,000 million years ago.

There are two fairly simple conclusions to be brought out of this: first, that orogenic belts do not necessarily form only during continent/continent collisions, and, second, that for much of the geological past a layer fraction of the continent of the world was not subject to rifting. This raises the intriguing possibility that it might be feasible to date (give or take a few hundred million years) the start of plate tectonics as it is known. Before that time one would have to assume that either plates as thin boundary layers did not exist or that somehow rifting was not a normal occurrence in tectonics. An amusing twist to uniformitarianism.

D. D.

HAEMOGLOBIN

Quaternary T, Tertiary r

from a Correspondent

INTEREST in the details of the structural differences between unligated high-spin deoxyhaemoglobin in the T (tense) state and the ligated oxy and met derivatives in the R (relaxed) state continues to stimulate further investigations. Anderson (*J. molec. Biol.*, **79**, 495; 1973) now describes a technique for obtaining crystalline methaemoglobin in the deoxy quaternary conformation; that is, with the tertiary structure in the met or r state, and with the quaternary structure in the deoxy or T state (the upper and lower case letters here denote quaternary and tertiary states respectively). As Anderson points out, a better stereochemical understanding of the pathway(s) by which conformational changes take place requires crystallographic studies of stable intermediates. Mutant human haemoglobins of the M type, in which the quaternary structure is locked because two of the four haems remain permanently in the met (r) state, have been useful in studies of the effect of ligand binding on the α or β subunits in the absence of a change in quaternary structure. Similarly BME-Hb, in which a bifunctional reagent has formed a covalent cross-link between the Cys(93) and His(97) residues of each β chain, is locked permanently into the oxy (R) state.

Anderson's new approach is to intro-

duce an acrylamide monomer into the water space of the human adult deoxy-Hb crystal by co-crystallisation, followed by photochemical polymerisation of the monomer inside the crystal, in the hope that the polymer would clamp the molecule in its original T quaternary structure, while allowing small, tertiary movements on ligand binding. Air oxidation of these deoxy-acryl crystals does indeed give met-acryl (T; t $\rightarrow$ r) crystals suitable for X-ray diffraction studies, in striking contrast to the breaking up of Hb-A crystals which normally follows changes in the ligation state.

X-ray analysis of these crystals to 3.5 Å resolution by the difference Fourier technique revealed marked changes in tertiary structure in the region of the haem pockets and the $\alpha_1\beta_2$ contacts between the subunits. The iron atom moves towards the plane of the porphyrin, though probably not the full distance of 0.45 Å which it moves in the deoxy (T) to met (R) shift. The even larger movement (nearly 0.75 Å) of the iron atom, almost into the haem plane, found for CO (R) haemoglobin cannot be observed in this constrained T state system because the crystals break up, presumably because of a transition to the R state, despite the presence of the polymer in the crystal lattice. Movement of the iron atom causes a change in tilt of the haem. This acts as a lever which initiates stereochemical changes weakening several hydrogen bonds, both within and between subunits, which determine the stability of the tertiary and quaternary deoxy structures. The later ligand of the r state ferric subunits causes a slight opening of the haem pocket in the α subunit and a much larger one in the β subunit.

The structural changes seen by Anderson in changing from the tertiary deoxy (t) to the aquomet (r) state within the quaternary T structure are similar but opposite to those seen by earlier workers in changing from the aquomet (r) state to the deoxy (t) state in the quaternary R structure of BME haemoglobin. Thus changes in tertiary structure associated either with addition of ligand to the T structure or the removal of ligand from the R structure are complementary. The electron density maps show that the α haems autoxidise more readily than the β haems, just as the β haems were reduced more easily than the α haems in BME haemoglobin.

Anderson's results support many of Perutz's earlier ideas about the mechanism of haem-haem interaction. They confirm that changes in tertiary structure on ligation are a consequence of the contraction of the iron-nitrogen bonds and also, especially in the β subunits, of the steric effect of the ligand itself. The differences in the roles of the two types of subunit contact are also evident. The $\alpha_1\beta_1$ interface, although showing

large collective movements, seems to be an essentially passive contact, as there is no evident set of movements linking α and β haems across it. By contrast, in the $\alpha_1\beta_2$ interface the smaller number of relative movements are concentrated on individual side chains. A series of interactions can be seen involving residues in van der Waals contact with α and β haems and mediated between subunits by a specific rearrangement of hydrogen bonds. Although the conclusions drawn from the X-ray study of the met-acryl (T, r) crystal must be regarded as tentative for several reasons, Anderson suggests that the major role of the $\alpha_1\beta_2$ interface in determining quaternary structure now seems to be well established.

BACTERIA

Computer Identification

from a Correspondent

TAXONOMY may be divided principally into classification, nomenclature and identification. Computerised classification based on the results of a large number of characteristics of individual bacterial strains has been developed, initially by Sneath. Taxonomic groups (taxa) are then chosen which contain bacteria with the greatest similarity of characters. Names are then assigned, according to an International Code, to the taxa. An unknown strain may then be identified by comparison with known, named taxa using a few diagnostic characters, often in the form of a key.

This identification stage has now been automated by the Computer Trials Department at the Central Public Health Laboratory, Colindale (*J. gen. Microbiol.*, **77**, 273; 1973). The department has been building up this system since 1967 and it has based its evidence on trials of 1,595 bacterial strains, 1,079 of which were reference strains and the remainder mostly strains sent for identification by the Public Health Laboratory Service. These were strains which the PHLS laboratories had had difficulty in identifying, and which a large centralized laboratory could more easily deal with.

The groups chosen as the known taxa in the system were made up from those bacteria known to be found in medical specimens, those strains considered pathogenic and a few other strains. The bacteria were all Gram-negative and rod-shaped. The forty-eight characters or tests chosen for the survey were those which were considered the most reproducible and easiest to prepare, carry out and read. The range of the tests chosen included many of the objective, biochemical tests used in medical bacteriology laboratories.

The method used for identification was polythetic—that is, organisms shar-

ing the most features were grouped together—and also simultaneous. An unknown strain was compared on the forty-eight characters simultaneously with all the stored taxa to obtain identification in one step. A table (matrix), which gave the probability of a strain, in any given taxon, giving a positive result in each of the tests, was first constructed. When all strains in a taxon were positive for any particular character, a value of 0.99 was allotted and a corresponding value of 0.01 was allotted when all results were negative. This prevents a single aberrant test result from precluding all possibility of identification with otherwise suitable taxa. Unknown individual strains were then tested and identified on the basis of the results.

The likelihood that any unknown strain is a member of a particular taxon has been defined as the probability that a member of that taxon would give the same results as those for the unknown. For each strain in the study to be identified, a computer printout was produced; this included reference data on the strain, a list of the tests performed and the results obtained; if the strain was successfully identified, a list of the likely taxa to which it belonged, ranked according to their identification scores, was printed. If a strain failed to identify, then a list of further tests, necessary for successful identification, was compiled.

The computer department's results show that about 90% of all the identifications agree with conventional methods; this demonstrates the potential success of the system, because many of the reference strains are unusual and the Public Health Laboratory Service strains were those which had already caused some difficulty. Some misidentifications which did occur were almost always due to the absence of taxa for identifying the unknowns, because there had to be a finite limit to the choice of known taxa in the matrix. For routine series of strains, that is, not selected because they are atypical, the authors believe that approaching 100% would identify correctly.

This study has shown the value of a central service, which can deal with rare and unusual strains in a quick, standard and reproducible way and to save time, labour and money. There is evidence that fewer tests need be performed than in conventional work. It has also demonstrated that a computer can store large amounts of data for identification and test selection, which is readily available as printout for detailed, cumulative reports. The potential of the system, perhaps by linkage to other automated apparatus, has yet to be fully realised, but, by itself, it is an essential taxonomic application.

IMMUNOLOGY

Tumours and T Cells

from a Correspondent

VARIOUS devices are known for the production and maintenance of mice which are numerically deficient in lymphocytes of thymic origin (T cells). Neonatal thymectomy was the first to be recognised but it has the disadvantage that the few babies which survive are often sickly. Congenitally athymic mice are currently popular but they are expensive and difficult to maintain. Mice thymectomised as adults and subsequently irradiated have often been used, however, and their viability is usually satisfactory. Woodruff and his colleagues now describe (*Proc. R. Soc. Lond., B184*, 97; 1973) the use of such deprived mice to explore the nature of antitumour immunity.

Three transplanted tumours were used. The first, a CBA-strain sarcoma, was grown in normal and deprived CBA mice. Its growth rate was slower in deprived than in normal mice. This implies that there was no effective resistance to tumour growth on the part

of the host which involved 'T' cells. Furthermore, injection of heat-killed *Corynebacterium parvum* inhibited tumour growth in both groups of mice. The authors conclude that in this instance whatever resistance to tumour growth there was had been exerted by macrophages stimulated by the adjuvant. The second tumour was an A-strain mammary carcinoma grown in normal and deprived A-strain mice. Again growth in the deprived mice was if anything slower than in normal animals and again *C. parvum* had an inhibitory effect on tumour growth in both groups of animals.

The third tumour was an A-strain fibrosarcoma which was grown as an allogeneic transplant in normal and deprived CBA mice and as a syngeneic transplant in A-strain mice. (It would have been of interest to have a record of the growth of this tumour in deprived A-strain mice.) It grew slowly but progressively as an allotransplant in normal CBA mice but rapidly in deprived CBA animals. Its growth rate in deprived CBA mice was equivalent to its growth rate in normal A-strain mice. No record is made of any attempts to influence the growth rate

Actin and Myosin in Non-muscle Cells

IN spite of its undoubted importance, the tale of actin and myosin in non-muscle cells has been over-long in the telling. What was new and exciting in amoeba and platelets has become progressively commonplace, as the list of animal cells to yield myosin-like ATPase activities or decorated filaments has grown. Moreover, the proteins themselves, with one conspicuous exception, have turned out to be strikingly similar to their counterparts in muscle cells.

So it may be with some hope of variety that biochemists are now turning to the other components of muscle. Proteins such as tropomyosin, troponin and actinin, which are present in the myofibrils and which regulate or hold in place the contractile events, could all have in non-muscle cells a relative which helps to harness the same force-generating machinery to the particular requirements of the cell.

Not surprisingly, the first of these proteins to be looked for is tropomyosin; a distinctive protein whose ease of purification and remarkably high content of α helix made it an early favourite with X-ray crystallographers. In muscle, this protein is thought to provide rigidity to the actin filaments and to mediate the regulation by calcium of the actinomyosin interaction. Neither of these functions is obviously necessary to other systems, and so it was interesting to learn, a year ago, from Cohen and Cohen (*J. molec. Biol.*, **68**, 383; 1972)

for blood platelets, and now from Fine *et al.* for embryonic brain and neurones (see *Nature New Biology* next Wednesday (October 10)), that these tissues also have a form of tropomyosin.

The new proteins resemble muscle tropomyosin in most of their properties—notably in their high content of α helix and consequent resistance to denaturation, and in their ability to function in the calcium regulation of muscle—but they differ in one important respect. The length of the rod-shaped muscle tropomyosin molecule as gauged from its periodicity in paracrystals is about 400 Å, which is long enough for it to lie in the groove of an actin filament alongside seven subunits. The new proteins have both lower molecular weights (30,000 as opposed to 35,000) and reduced periodicity in paracrystals (340 Å) so that, other things being equal, they are able to span only six actin monomers.

The finding of non-muscle tropomyosins, of course, raises more questions than it answers. What most cell biologists will hope not to see, after the experience with actin, is an extensive series of papers which show the presence of tropomyosin-like molecules in every conceivable cytological niche. It would be best to assume that a protein that is present in both blood platelets and nerve cells is likely to be of widespread distribution, and concentrate on the important question of what it does in the cell.

of the A-strain fibrosarcoma with *C. parvum*.

These simple experiments illustrate that tumours growing in their host of origin do not necessarily engender that immunological antipathy in the host lymphoid system which can easily be demonstrated against malignant foreign grafts. Furthermore, the adjuvant bacterium, *C. parvum*, need not necessarily be thought of as a T-cell stimulant.

CELL BIOLOGY

Tubules and Filaments

from a Correspondent

BIOLOGISTS with a wide range of backgrounds met to discuss the structure and function of microtubules and microfilaments at the York meeting of the British Society for Cell Biology on September 20 and 21. A. Forer (York University, Ontario) opened the meeting by presenting evidence that actin-like microfilaments interact with microtubules and speculated that these two components act like 'muscle' and 'bone' respectively. He drew attention to the clear zone around the outside of microtubules, and this feature was also present in the electron micrographs of several other speakers. Very little seems to be known about the structure and function of this zone, but it seems to be of sufficiently regular appearance to merit more study.

At the biophysical level, there seemed to be reasonable agreement between several speakers (D. Chasey, University of Edinburgh, E. O'Brien, King's College, London, L. Amos and R. Linck, MRC Laboratory of Molecular Biology, Cambridge) that, at least in the case of some ciliary microtubules, the structure is built up from a 3 start, 13 fold, left handed helix and it seems from preliminary evidence of O'Brien that *in vitro* reconstructed microtubules may have a similar structure. The study of the ultrastructure of microfilaments is technically even more difficult than that of microtubules, but D. Gilbert (King's College, London) demonstrated that neurofilaments from a marine worm are protein α helices supercoiled into several higher order helical forms. Research on microfilaments is impeded because of the lack of a specific disruptive agent equivalent to colchicine in the study of microtubules. Early optimism of some workers that cytochalasins may have specific effects on microfilaments seems to be fading, to judge by the discussion after a contribution from J. C. Appleton and R. B. Kemp (University College of Wales, Aberystwyth) showing effects of cytochalasin A on cell aggregation.

M. Jacobs (King's College, London) described a careful study of the exchange reactions between the two nucleotide binding sites on the tubulin dimer; the significance of transphosphorylation

between the sites during microtubule assembly does not seem clear. W. W. Franke and J. Stadler (University of Freiburg) have extended their work on colchicine-binding properties of membrane fractions and find that such binding may be distinguished from that of tubulin on several grounds, including binding of lumicolchicine.

Several workers approached the problem of interaction between microtubules. J. Cachon (Villefranche-sur-mer, paper read by M. Sleight) provided a possible explanation of complex microtubule patterns in the axopods of radiolarians in terms of different tubules being 'divalent' or 'trivalent' in their binding properties for other microtubules. J. Tucker (University of St Andrews) extended this discussion by speculating on the roles of templates and nucleating sites, as well as self assembly links, in the formation of microtubule arrays. Several factors can be involved in one organelle. A. C. Crossley (University of Sydney) presented evidence that microtubules can interact over considerable distances (around 800 Å) during myoblast development.

The potential value and difficulties of genetic approaches to microtubule formation were reviewed by J. R. Warr (University of York), who argued that mutants resistant to anti-mitotic agents may provide useful tools for such studies. In contrast with the situation in mammalian cell cultures, colchicine resistant mutants in *Chlamydomonas* seem not to be simply mutants with permeability defects and consequently may have microtubule defects.

Two botanists, A. W. Robards (Uni-

versity of York) and L. Goosen-de-Roo (Leiden), were in broad agreement with Forer who earlier suggested a structural rather than a contractile role for microtubules. Goosen-de-Roo presented a careful series of electron micrographs which support the proposition that rows of microtubules act during thickening of tracheary elements by forming barriers to guide vesicles to required locations.

PALAEOBOTANY

Flora of Siberia

from our Soviet Correspondent

THE first detailed results have been published (*Dokl. Akad. Nauk SSSR*, **209**, 1464; 1973) of the pollen and spore content of the stomach of the frozen remains of a horse found in a mine of the Selerikan basin in Yakutia (Siberia) in December 1968. The remains were dated to an absolute age of 37,000 years by the Laboratory of Absolute Geochronology at Leningrad University, a date confirmed by a control determination by Professor I. Harrington of the National Museum, Ottawa.

The pollen and spore analyses were carried out at the Komarov Botanical Institute of the Academy of Sciences of the USSR. Although further, more detailed, pollen analysis is still in progress, results to date show that the Selerikan horse may prove as significant for an understanding of the palaeobotany of Siberia as did the Beresovka mammoth. Like the Beresovka find, the Selerikan horse reveals an ecological picture typical of present-day Siberia. So far, seventy-two different types of

X-ray Structure of Antibody

THE first X-ray structure determination of the Fab' portion of an immunoglobulin of known antigen specificity has been accomplished by Padlan, Segal, Spande, Davies, Rudikoff and Potter at a resolution of 4.5 Å and appears in *Nature New Biology* next Wednesday (October 10).

As with chymotrypsin, lysozyme, and so many other proteins, it transpires once more that the molecule is made up of two globular halves of roughly the same size, each of them in this case being in turn subdivided into two domains. This of course accounts well for the four structural elements known to make up the Fab' fragment, namely the variable and constant parts of the light chain, and the corresponding variable and constant parts of that portion of the heavy chain (Fd') with which the light chain makes contact. These four parts can be regarded as centred on the vertices of an elongated tetrahedron, with dimensions of 40 × 50 × 80 Å.

The other interesting result, which

emerges from difference Fourier maps, is the location of the binding site of phosphorylcholine, the specific hapten of this myeloma immunoglobulin. Competition experiments have allowed the authors to distinguish between a non-specific site, located on the surface of the molecule in the constant region, and the true antigenic binding site. Again, as repeatedly found in enzymes, the striking feature of the specificity site is its location at the bottom of the deep cleft which divides the molecule into its domains. In the case of the immunoglobulin the site is sandwiched by the two masses making up the variable region and formed by elements of both light and heavy chains.

The absence of any important features in the difference map, other than the peaks corresponding to the ligand itself, is taken to indicate that the attachment of the hapten is not accompanied by any large conformational readjustments, such as occurs in allosteric species as, for example, haemoglobin.

pollen have been identified, predominantly herbiage (Graminae, Cyperaceae, Caryophyllaceae, *Ranunculus*, Rosaceae and Compositae) amounting to 80.9% of the whole, with smaller quantities of spores (11.3%) and the pollen of shrubs and trees (7.8%). Taking into account the known feeding habits of horses, these results suggest a meadow-type terrain, interspersed with bushes and shrubs, the leaves and shoots of which were consumed together with the herbiage.

The presence of pollens and spores from different ecological groups suggests that shortly before its death the horse had been ranging over different types of habitat—dry regions, mesophytic meadowland and damp riparian areas. It is noteworthy that the pollen grains of all types of plants, both monocotyledons and dicotyledons, were well matured, indicating that the horse perished in late summer. Since the Beresovka remains suggest an early summer dating, a slightly different ecological pattern between the two finds, irrespective of place or date, is to be expected, due to seasonal variation, and it will be interesting to see what differences further analysis may reveal.

Almost all the plants represented by the Selerikan find are still indigenous to northern Yakutia. The exceptions were a few grains of conifer pollen, notably *Picea obovata*. It is very possible, however, that these represent wind-blown pollen from the south that had settled on the grass and been ingested with it, rather than a part of the local flora.

PHYSICAL METALLURGY

Order-disorder in Alloys

from our Materials Science Correspondent
THE order $\rightleftharpoons$ disorder transformation in alloys was the theme of an international symposium of metallurgists and physicists at Tübingen University from September 3–5. The proceedings, organised by Drs H. Warlimont and W. Pitsch of the Max-Planck Gesellschaft, were largely concerned with the behaviour of solid solutions such as Cu_3Au , Fe_3Al , FeCo , Ni_3Mo , CuPt , which share the characteristic that at high temperatures the atomic distribution is wholly or almost wholly random but becomes ordered below a critical temperature. The distinguishing feature of the occasion was the emphasis placed on the statistical mechanics of the ordering process and also on its crystallographic and metallographic details; on the other hand the resultant changes in properties, especially mechanical ones, occupied a subordinate role.

The central concept in terms of which the ordering transformation has long been analysed is the interaction energy

between first or second nearest neighbours, a variable which depends on the chemical identity of the neighbours. The interaction energies determine the extent of short-range ordering, the value of the critical temperature and the nature of the ordered "superlattice". Less directly, they determine ordering kinetics and also the form of the associated phase diagrams, that is, maps of the ordering behaviour of off-stoichiometric and two phase alloys. At the conference, substantial progress was reported on each of these fronts. Thus D. de Fontaine and H. Yamauchi (University of California, Los Angeles) were able to construct a novel theory of ordering kinetics in terms of pair-site probabilities (directly related to interaction energies), and a group led by W. Pitsch (Max-Planck Institut für Eisenforschung, Düsseldorf) has made much progress in predicting phase diagrams in terms of interaction energies. C. M. van Baal

(Delft University of Technology) is also effectively active in this field. Physicists would also like to be able to move backwards to even greater generality by understanding neighbour interaction energies in terms of electronic characteristics of the constituent atoms and of the elastic and bond-theoretical characteristics of the crystals, but on this very difficult cluster of problems little progress was reported, and indeed none is on the horizon. One feature which is becoming clear, though, is that two-particle interactions are too simplistic an approach. For instance, P. Clapp (Ledgemont Laboratory of the Kennecott Copper Corporation, Lexington) showed convincingly that the peculiar ordered structure in CuPt can only be understood in terms of four-atom group interactions.

A generalised theory of superlattice types, by A. A. Smirnov (Ukrainian Academy of Sciences, Kiev), was out-

More Suppressor T Cell Effects

SINCE the realization by at least some cellular immunologists that the sequence of cellular events following injection of an antigen should not be thought of as involving the linear differentiation of a single cell type, the fun has been fast and furious. Well-grown notions of six or seven years standing (the exact time depends upon the starting point) are those of T lymphocytes (of thymic origin) and B lymphocytes (not of thymic origin). Inevitably various categories of T and B cells have been proposed; the macrophage has been dragged in and real enthusiasts have sought to embrace the mast cells, eosinophils, and sundry other cells. One of the latest and more entertaining manoeuvres has been the creation of the suppressor T cell and this is the subject of a communication by Okumura and Tada in *Nature New Biology* next Wednesday (October 10).

Adult donor mice were injected twice with either sheep or horse erythrocytes. Two weeks after the second injection they were killed and their spleens and thymuses were prepared, separately, for injection into recipient mice. These recipient mice were immunised once or twice with heavily dinitrophenylated horse red blood cells (DNP-HRBC) plus a pertussis adjuvant. The transfer of cells from the donor mice into the recipients was made at the time of either the first or the second immunising injection of DNP-HRBC. It was found that when the donor cells derived from an HRBC immunised mouse—that is, the carrier which is homologous to the carrier of DNP—the anti-DNP response was much suppressed, whereas cells from the heterologous carrier (SRBC) immunised donors had little or no such suppressive effect.

It did not matter whether the suppressive cells were culled from the thymus or the spleen of donor mice but in either instance their inhibitory effect could be abolished by incubation with anti- θ antiserum and complement before transfer. The conclusion from this last procedure is that the suppressive effect is exerted by T cells. The authors remark that cells within the thymus are not normally regarded as susceptible of immunological activation. The specific suppressive effect of cells harvested from the thymuses of their donor mice does, however, suggest that in some circumstances the thymus should be thought of as directly involved in immunological responses.

The interest of experiments of this kind lies, first, in consideration of whether similar 'T' cell suppressor effects occur in intact animals during the course of less controversial immunological responses and, second, in speculation as to the identity of the suppressive mechanism. No evidence is offered in the present experiments as to how the injected donor cells responded to their third contact with HRBC but other work suggests that T-cell activation is a prerequisite of expression of suppressor function.

The present experiments of Okumura and Tada record a suppressive effect of T cells on B cells which is measured by an anti-hapten response and although it is not explicitly stated there was presumably a suppression of the anti-carrier response. In a recent issue of *Nature* (244, 195; 1973) other suppressed targets of activated T cells were alluded to. These cells certainly seem equipped to perform both positive and negative regulation of immunological processes.

lined in abstract in the programme: it seemed to be an interesting development of M. J. Richards and J. Cahn's recently published theory. The paper was, however, withdrawn and Smirnov did not come. Several other Soviet contributions were also withdrawn, but more serious—in view of the extensive and important Soviet involvement in this field of metallurgy—only one Soviet participant was able to join the conference early on; several others turned up on the last day, having thereby wrought havoc on the programme. This happened in spite of two years of painstaking correspondence by the organisers with the Soviet Academy of Sciences. It seems that even so august an official body as the academy carries no weight with the even more august functionaries who issue passports and thus dominate the movements of their compatriots.

The metallurgical and physical aspects of the conference converged in the session devoted to ordering mechanisms. The long standing debate between the adherents of nucleation-and-growth mechanisms and those favouring a homogeneous ordering mechanism was not brought any nearer to resolution by this occasion; but several interesting contributions (both experimental and theoretical), notably those by A. G. Khachaturyan (Institute of Metallography and Metal Physics, Moscow) and by P. Eurin and colleagues (Centre d'Etudes Nucleaires, Grenoble), dealt with the microstructure of alloys (such as CoPt) in which minute differently oriented domains are formed in such a way as to minimise internal stresses. The associated theory is also valid for age-hardening alloys containing Guinier-Preston zones, and interesting progress may be looked for here. Eurin's paper was one of a large number of French contributions which reflected the high level of experimental activity in France on various aspects of order in alloys.

Several interesting contributions were devoted to experimental study of ordering kinetics and the light cast thereby on ordering mechanisms. An unusual approach was that by D. Morris, F. Besag and R. E. Smallman (University of Birmingham), who, by an ingenious procedure, have studied the disordering characteristics of the classical alloy, Cu₃Au; their approach allowed degree of order and domain size to be varied independently.

Perhaps the most novel part of the conference was the session devoted to order in interstitial alloys. In particular, M. Hirabayashi and colleagues (University of Tohoku, Sendai) presented full experimental findings on solutions of O, H and D in Ti, Zr and Hf. Over broad composition ranges, there are multiple stages of interstitial ordering as the temperature is lowered. M. A. Pick

and colleagues (Institut für Festkörperforschung, Jülich) presented a thorough experimental study of interstitial ordering of hydrogen in niobium. These two investigations in turn related to studies by S. Iijima and J. M. Cowley (University of Arizona) on ordering of planar defects ("shear planes") in off-stoichiometric transition metal oxides: it is just beginning to be recognised that this ordering is a function not only of composition (that has been recognised for several years) but also of temperature. The drawing of parallels between previously disparate fields, such as interstitial ordering and shear-plane ordering, was one of the achievements of the conference. Just about the only form of ordering not covered was that in organic systems, such as the molecular ordering in anthracene-based solid solutions, recently discovered by P. M. Robinson and H. G. Scott (University of Melbourne).

THE EARTH

Bumpy Asthenosphere

from our Geomagnetism Correspondent

In the days when only a few geologists believed in the large-scale horizontal movements implied by continental drift, their colleagues could nevertheless quite happily accept that large areas are uplifted and subside from time to time. To most people, the concepts of vertical and horizontal motion were

independent; and there seemed to be no inconsistency in accepting one while denying the other. Indeed, some of the Earth scientists who still do not accept the tenets of plate tectonics go so far as to claim that the vertical movements of continents are incompatible with drift. This is, of course, an extreme and minority view; yet even most drifters would probably suppose vertical and horizontal motions to be unrelated, because it has generally been assumed that plates move over an asthenosphere with a level or constantly sloping upper surface.

But this last assumption has now been questioned by Menard (*J. geophys. Res.*, **78**, 5128; 1973), who argues instead that the surface of the asthenosphere undulates. One consequence of this is that as the lithosphere drifts over the asthenospheric 'bumps' it will rise and fall. The vertical movement of large areas of continent and ocean floor thus becomes a necessary result of horizontal motion; and Menard suggests that the absence of epeirogenic uplift, subsidence, normal faulting and volcanism would even be reason enough to question the reality of lithospheric drift.

The idea of a bumpy asthenosphere is not strictly new with Menard. According to Faure (*C. r. hebdom. Séanc. Acad. Sci., Paris*, **D265**, 3239; 1971), for example, the African sedimentary record suggests the passage of an epeirogenic wave of height 1–10 km, wavelength 100–2,000 km and period 10^7 – 10^8 yr

Ribosomal Genes in Species of Bean

It is well known that in higher plants there is a wide variation in the amount of nuclear DNA, even after corrections for polyploidy. In the genus *Vicia*, for example, *Vicia faba* has between five and seven (according to report) times as much nuclear DNA as *Vicia sativa*, although they have the same diploid number of chromosomes. Two models attempt to explain how this extra DNA is arranged within the chromosomes; one suggests that the DNA increases in bulk by the thread becoming longer, and the other explains the increase by parallel (lateral) expansion, resulting in multistranded chromosomes. There is good evidence for both models which are not, in any case, mutually exclusive. The lateral multiplication model makes the prediction that a two-fold (say) increase in DNA should result in a two-fold increase in gene dosage.

In *Nature New Biology* next Wednesday (October 10), Maher and Fox test this prediction by measuring directly the amount of DNA in different species of *Vicia* and correlating it with the number of ribosomal genes (rDNA) as measured by hybridisation techniques. They find that as the amount of DNA increases so does the amount of rDNA, but not

in direct proportion, suggesting that DNA is not increased by lateral multiplication. But when only the relative amounts of DNA in the *Vicia* species are considered they approximate to a ratio of 1:4:8, compatible with DNA increase by discrete strand formation. Thus the authors present experimental results for and against the model of DNA increase by lateral multiplication, but taken with other evidence in the literature, their preferred model is longitudinal multiplication.

Maher and Fox have also measured the amount of rRNA synthesized by the four species of *Vicia* to see if they can be correlated with the number of ribosomal genes. Since little is known about rRNA gene utilisation in plants, gene activity has been estimated by measuring the volume of the prophase nucleoli. This technique revealed that large nucleoli were associated with an increased number of rRNA genes, but, here too, not in direct proportion. The authors conclude that as the total amount of DNA increases within the *Vicia* species considered, less of the genome is transcribed, so that proportionally fewer ribosomal genes are needed in translation.

with a velocity of $0.1\text{--}10\text{ cm yr}^{-1}$ since the Cretaceous. Faure notes that although this could be a wave in the asthenosphere, the same result would be achieved by the lithosphere moving over the undulating asthenosphere. Burke and Wilson (*Nature*, **239**, 387; 1972) have also described epeirogenic movements in connection with rising mantle plumes. But these examples relate to relatively localized regions. What Menard has now done is to investigate the possibility of asthenospheric bumps over much larger oceanic areas.

So how many asthenospheric bumps be shown to exist? The principle of Menard's method is simplicity itself; bumps will affect the relief of the ocean floor, and so depth contours should reveal the bumps directly. Unfortunately, factors other than the supposed bumps also govern ocean floor relief. For one thing, depth will depend on the density and thickness of the oceanic crust and lithosphere. But these lithospheric effects may be assessed independently, for average depth is a function of age and is explicable in terms of the shrinking of a cooling lithosphere. Thus in an area where magnetic anomalies, and hence ages, have been mapped and where the empirical relationship between age and depth is known, it is possible to derive a map of 'standard' depths for an ocean floor uncovered by sediment. Subtracting the standard depths from the observed depths (corrected for sedimentation and high frequency topography such as ridges and volcanoes) then results in a depth anomaly map in which positive anomalies represent depths shallower than the standard depths (elevation) and in which all anomalies may hopefully be attributed only to the effects of asthenospheric relief.

In the northeastern Pacific, the region investigated in most detail by Menard, there are apparently two scales of anomalies. In the north there is an irregular but persistent positive anomaly of $300\text{--}700\text{ m}$ having dimensions greater than 600 km by $2,000\text{ km}$, and in the southwest there is a second large-scale positive depth anomaly of $100\text{--}300\text{ m}$. Between these two the anomalies range from $+300\text{ m}$ to -400 m but are negative on average, giving effectively a large negative anomaly about $1,000\text{ km}$ wide and at least $5,000\text{ km}$ long in the northeasterly direction. Superimposed on these large-area anomalies are smaller ones having values of about 300 m and linear dimensions of $500\text{--}1,000\text{ km}$. That there is little or no systematic error involved in the procedures by which the anomalies were derived is shown by the fact that positive and negative each occupy about half the area and by a lack of correlation between the sign of the anomalies and crustal age, depth, or sediment thickness.

The depth anomalies plotted by Menard correlate with neither crustal thickness nor heat flow, cross fracture zones in spite of regional changes in depth, extend across magnetic anomalies and active spreading centres, and thus seem to be unrelated to either old or new plate boundaries. But they do seem to correlate weakly with gravity anomalies of the same sign—a phenomenon which Menard relates to the effects of mantle convection and hot spots. It has been known for some time that the broad gravity anomalies of wavelength $1,000\text{--}2,000\text{ km}$ mapped by satellite cannot be attributed to the lithosphere, which is incapable of supporting elastically features of such dimensions, and must therefore reflect flow in the mantle. The correlation of broad gravity anomalies with asthenospheric undulations thus implies that the undulations also result from convection in the mantle.

More specifically, positive gravity anomalies and positive depth anomalies are associated with rising convection; and, of course, hot spots are forms of rising convection. Indeed, Morgan (*Bull. Am. Ass. Petrol. Geol.*, **56**, 203; 1972) has already shown that hot spots are generally associated with positive gravity anomalies. Menard thus concludes that asthenospheric bumps are as fixed as hot spots and must persist for at least as long, although it must be said that he also adduces evidence for the persistence of asthenospheric bumps from the history of the elevation and subsidence of oceanic islands. In view of the widespread current interest in hot spots, Menard's views on the rela-

tionship between hot spots and asthenospheric bumps will no doubt attract considerable attention. But this should not be allowed to obscure his more important general point that "many aspects of vertical tectonics are necessary consequences of plate tectonics on a bumpy asthenosphere".

ENGINEERING GEOLOGY

Reclaiming Land

from a Correspondent

THE Regional Meeting of the Engineering Group of the Geological Society, held at the University of Durham from September 10–13, was introduced by R. K. Taylor (University of Durham) who drew an analogy between the viability of colliery discards as engineering fills (4 million and 2 million tons per year in Durham and Northumberland, respectively) and the two "waste" Pennine gangue minerals of past centuries. Fluorite and barytes had subsequently become attractive propositions to the steel and drilling industries, respectively.

D. Blakeway (Binnie and Partners) spoke about the stability of spoil heaps and hillsides in South Wales. Investigations since Aberfan have revealed exceptional hydrogeological factors in the coalfield with up to 40 feet of mining subsidence and rainfall figures of between 60 and 95 inches per year. On a local basis the Reclamation Officer for Co. Durham (R. A. Briggs) said that reclamation was now outpacing dereliction in the county, the predominant use of reclaimed spoil heaps being agricul-

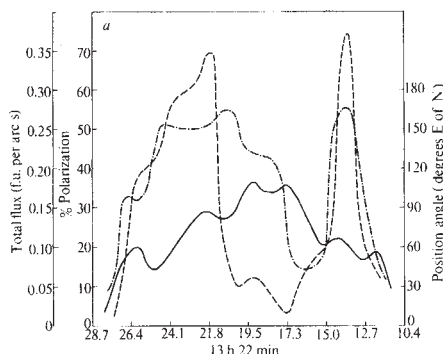
Centaurus A studied at 10.7 GHz

CENTAURUS A (NGC5128) is the nearest strong radio galaxy to us, just 4 Mpc from our galaxy. That makes it a prime target for radio astronomers interested in the structure of such objects, since it will be possible to resolve finer details in Cen A than in any comparable object. In *Nature Physical Science* next Monday (October 8) Price and Stull report high resolution observations of the

galaxy made at 10.7 GHz during June, July and August of this year with the five element array of the Stanford Radio Astronomy Institute. This gives, at that frequency, a resolution of 17 arc s.

There are three lobes making up the radio counterpart of NGC5128, and each was scanned during these studies. Both the northeast and southwest lobes have themselves been resolved into more than one component (see figure), and the data are by and large explicable in terms of synchrotron emission from several compact regions.

Price and Stull speculate on the basis of this evidence that other radio galaxies might also contain small-scale structure which includes both transparent, strongly polarised components and opaque, weakly polarised components; thus the overall low polarisations observed are an artefact of inadequate resolving power. This speculation is in line with the view that objects like Cen A are the later stages of QSOs and compact radio sources in galaxies, where repeated outbursts might be associated with ejection of clouds of relativistic electrons.



Southwest lobe of Cen A, observed at 10.7 GHz. —, Total flux; ---, % polarisation; - · -, position angle.

ture (50%) and forestry (34%). Figures produced by S. W. Pardoe (National Coal Board Opencast Executive) implied that coal from opencast mining is currently about £2 per ton cheaper than deep mine coal and that in this field again the sites can be advantageously restored, mainly to agriculture. A. D. M. Penman (Building Research Establishment) pointed out, however, that the volume of world mining is doubling every 14 years and the demand for construction on all types of previously opencast land has led to a joint research programme with the Coal Board. Discussion from the floor disclosed that there is no stability relationship between age and depth of backfill. Moreover, ingress of water is commonly believed to induce the sudden settlements recorded on some backfilled sites and Penman suggested that flooding might be one method of improving compaction.

With respect to site investigations in redevelopment areas both K. W. Cole (Ove Arup and Partners), who cited case histories to emphasize problematical foundation situations in glacial deposits and areas of shallow mine workings in the North-East, and M. I. Beeby and L. Threadgold (Exploration Associates Ltd) made a plea for improved procedures and organization. W. R. Dearman (University of Newcastle upon Tyne) considered that geotechnical maps could lead to more rational site investigations and demonstrated several types drafted from collated geotechnical information pertaining to Tyneside.

Coastal reclamation was brought into sharp focus by G. B. M. Oliver (National Ports Council) who explained that because the parts are required to operate commercially there is no great incentive to utilize maintenance dredgings for land reclamation; disposal at sea is usually cheaper. Emplacement of dredging muds from estuaries was discussed by P. R. Vaughan (Imperial College, London) who showed the value of under-drainage and controlled drying. He forecast that hydraulic fills of this type could produce in less than a decade usable ground no worse than many existing alluvial deposits. Other speakers, including A. C. Meigh (Soil Mechanics Ltd), advocated dynamic compaction for granular soils down to coarse silt size as well as for other types of fill. This technique involves dropping a heavy weight on to the ground surface from a great height.

Feasibility studies for a number of coastal reclamation schemes including Maplin were described, and T. D. Ruxton (Binnie and Partners) mentioned that the long period required for the evaluation of some schemes in Britain was often due to hydraulic seepage problems. G. G. Pickton (Department of the Environment) remarked that a

scheme involving the reclamation of 30,000 acres at Maplin had been formulated in the eighteen fifties at a cost of £3,738!

E. L. Streatfield (Cremer and Warner) in his introduction to the session on groundwater pollution said that the proportion of indisputable toxic industrial waste is just over 2%, and a further 5% may fall into this category. Preliminary results from a multi-authority review of 2,494 landfill sites in England and Wales were presented by D. A. Gray and J. D. Mather (Institute of Geological Sciences). This desk study has revealed that only fifty-one sites present a risk to aquifers and of the 714 locations examined by the river authorities only eighty were classified as unacceptable.

NUCLEAR PHYSICS

Reaction Times

from our Nuclear Theory Correspondent

It is usual to divide nuclear reactions into two stages: the first is the direct interaction of the incident particle with the target nucleus, and this takes place in the time it takes for the particle to cross the nucleus, usually about 10^{-22} s. After the direct stage, the remaining nucleus is in an excited state in which the energy is shared statistically among all its nucleons. Many interactions can take place among them, and eventually it happens that a nucleon or group of nucleons near the nuclear surface receives enough energy for it to escape, and this continues until the remaining nucleus is left in its ground state. This process is the decay of the compound nucleus, and takes a much longer time, of the order of 10^{-14} to 10^{-18} s.

The theories of the direct and compound stages of the nuclear interaction are now quite well understood, and it is possible to calculate the angular and energy distributions of the particles emitted in each stage, and to compare their sum with the experimental observations.

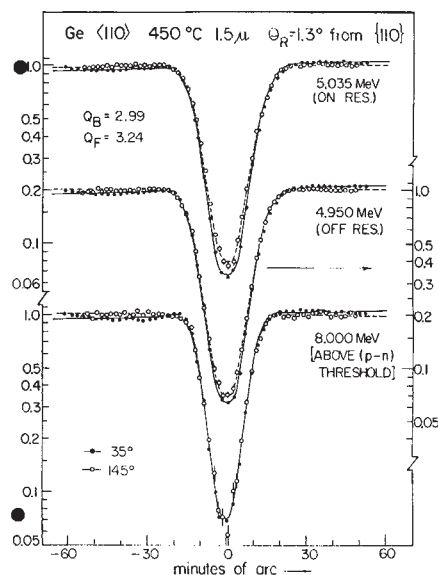
The time delay between the two processes naturally raises the question whether they can be observed individually, but because the most refined electronics cannot resolve times shorter than about 10^{-9} s, it is quite impracticable to do it directly. A very ingenious way of doing this indirectly has, however, been developed recently, and Professor G. M. Temmer and his colleagues at Rutgers University and Bell Laboratories reported at a recent conference that they have used it to resolve the direct and compound contributions to the elastic scattering of protons by germanium-72 (*Proc. int. Conf. nucl. phys., Munich, August 27–September 1, 1973; North-Holland, Amsterdam, 1973*).

The method depends on using a crystal as a target so that the nuclei are

in a regular spatial array. If a nucleus in a regular crystal emits a particle along the direction of one of the crystal axes, it will collide with the next nucleus and be scattered. Thus particles emitted in these directions are blocked and cannot escape from the crystal. If, however, the emitting nucleus moves from its original position, a particle emitted along the crystal axis will travel between the lines of nuclei and so escape. A struck nucleus recoils and the distance it travels before emission depends on the time between the initial impact and the decay; this is different for a direct and a compound process. In a direct process the target nucleus does not have time to move, but in a compound nucleus process it may move sufficiently far from its equilibrium position to increase the chance of escape of a particle emitted along a crystal axis.

The figure shows the intensities of particle emission as the detector moves across the line of the crystal axis, and the dip due to blocking is clearly seen. This was done for emission angles of 35° and 145° , and a small difference between them can be seen. The direct process contributes proportionately more at the forward angle of 35° than at the backward angle of 145° , and the amount of this difference allows the mean lifetime of the compound nucleus to be determined; the result is found to be about 1.4×10^{-16} s. The three sets of measurements illustrated refer to energies on and off the isobaric resonance at 5 MeV and at an energy above the (p,n) threshold. At this last mentioned energy so many reaction channels are available for the decay of the compound nucleus that the compound elastic process is negligible. Thus the direct reaction dominates at both angles, so the transmission curves are the same.

This very ingenious measurement makes possible a series of refined tests of nuclear reaction theories in an entirely new way and marks a notable advance in nuclear physics techniques.



Organization of Science and Technology in Japan

Y. KAWASHIMA

Nuclear Material Control Centre, Akasaka Park Building, 2-3-4, Akasaka, Minato-ku, Tokyo

In this article Dr Kawashima traces the development of science policy in Japan and assesses the prospects for the future.

SCIENCE and technology increase in importance as industry grows and becomes more sophisticated. Once regarded as something of a magic wand, it is now the subject of concern because of the fear that it may lead to a disastrous world. In this context I shall evaluate the effectiveness of science policy in Japan and predict its future development.

Growth of Central Machinery

The merits of the establishment of central machinery in the field of science and technology are still the subject of world-wide debate, but in Japan such a central organization was formed in 1956. The Science and Technology Agency came into being as the result of more than five years of continuous efforts by people who were enthusiastic about creating an administrative nucleus for science and technology.

The principal function of the agency was to coordinate the activities of all the ministries concerned with the development of science and technology. In order to plan, coordinate and promote, the agency's administrative structure initially included the Planning and Coordination Bureau, the Research Promotion Bureau, the Resources Bureau and the Minister's Secretariat. But it so happened that the Atomic Energy Bureau (AEB) had been established at the beginning of 1956 and it was decided that when the agency was formed it would also incorporate the AEB. This decision greatly influenced the development of the agency as will be seen later. The agency opened its office in May 1956 with a staff of 250 and a budget of 2,000 million yen (£2.8 million).

One of the first research institutes to work with the agency was the National Aerospace Laboratory, which had been established in 1955. Two months after the creation of the agency, the National Research Institute for

Metals was formed and also came within the agency's ambit of supervision.

The development up to the present day can be seen in Fig. 1. The names of the bureaux have changed slightly but the same functions are encompassed. Now, after sixteen years, the agency's family includes seven special corporations (set up by specific acts) and six research institutes, all of which are under the jurisdiction of the Minister for Science and Technology. Four of the organizations are in the field of nuclear energy, two in the field of space and five are undertaking programmes which serve as a basis for research in government and industry. The remaining two, the Japan Information Centre for Science and Technology and the Research Development Corporation of Japan, are unique organizations for promoting science and technology in Japan. The agency is now responsible for more than 7,000 persons and has a budget of nearly 89,000 million yen (£127 million).

Shifting Direction of Science Policy

In 1959 the Council for Science and Technology was established to serve in an advisory capacity to the Prime Minister, who acts as chairman. The agency serves as the secretariat. In 1960 the council produced its first report, "Comprehensive and Fundamental Measures for the Development of Science and Technology for Ten Years Ahead", which made broad recommendations but emphasized the need to increase the level of investment in research and development in Japan as a whole. A target of 2% of the national income was set for 1970, which was rather ambitious when compared with the corresponding figures in advanced countries (for 1958 some of the figures are 2.7% (USA), 2% (Britain), 1.8% (France)) but the target was reached in a surprising way.

From Table 1 it can be seen that even though the actual national income far exceeded the forecast of 1960, the investment in research and development still reached 2%; perhaps the rapid growth of the Japanese economy facilitated the achievement of the target.

Analysis of the figure of 2% shows that in 1970 the government share of expenditure on research and development was 25.2% while industry and other private organizations contributed the remaining 74.8%. The division is characteristic of the 1960s and contrasts sharply with con-

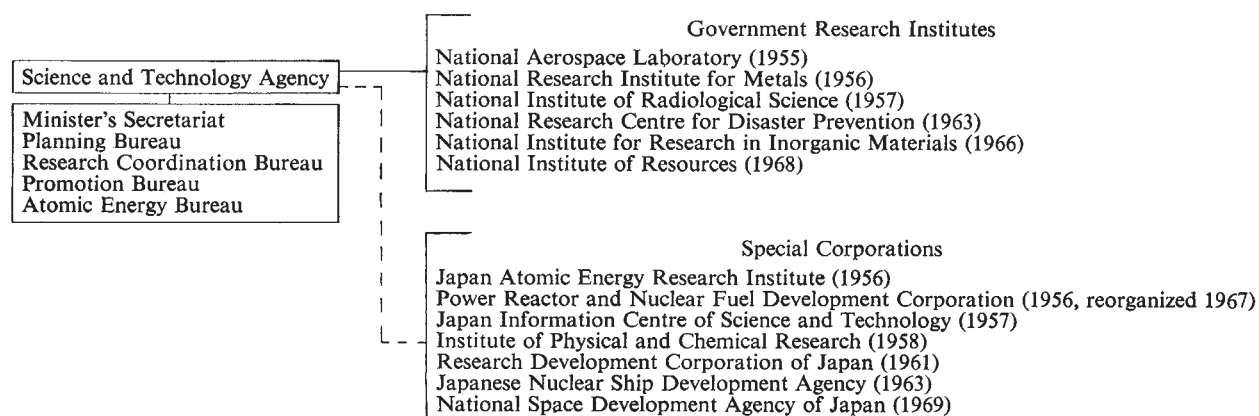


Fig. 1 Responsibilities of the Science and Technology Agency. Year of establishment is shown in parentheses.

ditions in the United States and Britain. In Japan, in 1970, only 6% of government research and development funds were transferred to industry, research institutes, and privately owned universities.

Fields of Contribution

In this framework the government took steps to increase expenditure on research and development by itself on the one hand, and to give incentives to industry on the other. The latter were provided in two ways: subsidies or grants for research and development in industry and financial incentives, including loans by governmental banks and tax exemption.

Table 1 Data for 1970

	National income (A) Thousand million yen	Investments in research and development (B)	B/A
Forecast made in 1960	21,243	425	2%
Actual	59,048	1,195	2%

Although the incentives provided by the government may guide industry in the desirable direction, it may be wrong to assume they have made a substantial contribution to the growth of research and development in industry. Generally speaking, industry put its efforts, most successfully, into applying research and development to increase production.

The ratio of the payment for technology introduced into the country to that of expenditure on research and development was 20:80 in 1961 and 14:86 in 1970. The period 1961-70 was one in which Japanese industry had to overcome drastic changes in its structure by assimilating sophisticated technology from abroad. Much of the effort on research and development was probably devoted to bringing the introduced technology into industrial production and to improving the technology.

By contrast, the government weighted its contribution towards promoting scientific research in the universities by allocating half its expenditure on research and development to this end. This resulted in the production of numerous scientists and engineers of high quality, so increasing the capability of exploring new scientific fields and of digesting and improving introduced technology. Thus the government's contribution was to facilitate economic progress in the long term.

Looking at the overall picture of science and technology in the 1960s it may be said that emphasis was placed on increasing efficiency of industrial production. The first white paper on science and technology published by the agency in 1958 in fact stressed the need to promote science and technology for the purpose of improving the balance of payments by reinforcing export industries. The success of the policy was reflected in the rise of the income per capita in Japan from \$US300 in 1958 to \$1,600 in 1970. But the adverse effects of industrial development became apparent and from about 1970 the population began to reassess the situation and to look forward to a future which they felt should be modelled along different lines.

Future Policy

A shift of emphasis in science policy could be detected in a white paper published by the agency in 1970, and in April 1971 the council published concrete recommenda-

tions in the report "Fundamentals of Comprehensive Science and Technology Policy for the 1970s". Although the report lifted the target of investment in research and development from 2% to 3% this was not the major concern; instead more emphasis was placed on the contribution of science and technology to social rather than economic development.

The report touched on three aspects, the first of which was the necessity to define as precisely as possible the needs of society in the future. Second, it dealt with the introduction of methods of technology assessment in order to steer science and technology in a desirable direction. Third, it spelled out three priority areas for research and development in the 1970s; the environment, life science and "soft" science. "Soft" science, a somewhat ambiguous concept, reflects the importance of a new multidisciplinary approach to such complex problems as urban problems and health care. The report, which is the outcome of joint efforts by the council and its secretariat in the agency, forms the basis of planning for science and technology in Japan in the 1970s.

Budgets

What is the structure of the government research and development budget? There are two categories; one is the Expense for Promotion of Science and Technology which includes the operating expenses of government research institutes, subsidies and grants to industry and special corporations, administrative expenses, plus the expenditure on space and nuclear energy. These areas are coordinated by the agency.

The second category is Expense Related to Promotion of Science and Technology and this encompasses the one mentioned above but also includes research expenses for universities (the chief portion) and expenditures on research and development included in some of the projects of various ministries; it thus represents all research and development funded by the government.

The relation between the two categories is shown in Table 2.

Table 2 Government Research and Development Budget

	1961	1970	1972
Expense for Promotion	28	114	168
Expense Related to Promotion	63	263	374

Units of 1,000 million yen.

The details of the Expense for Promotion budget for fiscal year 1972 are given in Table 3. Three points are clear: (a) the agency accounts for more than half the total amount, the Ministry of International Trade and Industry for 17% and the Ministry of Agriculture for 12%; (b) the proportions for nuclear energy (30%) and for space (1.2%) are extremely high; (c) government research institutes account for nearly 30% of the total.

Coordination

Coordination is carried out primarily by discussion before formulation of the budget. The agency goes over the programme with the organization concerned and indicates guidelines which are of a very general nature because the selection of specific research topics depends on the initiatives of the researchers. The agency specifies which are to be regarded as the priority subjects and recommends the

level of expense per researcher. (One of the striking successes of the agency has been its ability to rationalize the submissions of the organizations to the Ministry of Finance. As a result the funds allocated on a per capita basis have steadily increased.) After the agency has given its advice the organization prepares its budget and submits it directly to the Ministry of Finance. Separately the

each is allowed more autonomy within the framework of the agency. In effect the agency, as it coordinates the research and development of all ministries concerned, is able to foster a "new baby" as a priority and bring it up within the agency by providing special status.

The reason why these measures have proved feasible is that recently most of the priority areas have required a

Table 3 Expense for Promotion of Science and Technology

	Government research institutes	Subsidies and grants	Administrative expenses	Space	Nuclear energy	Total
The Diet	—	—	111	—	—	111
Prime Minister's Office	8,016	6,580	2,071	19,811	55,884	92,364
National Police Agency	337	—	—	—	—	337
Hokkaido Development Agency	95	—	—	—	—	95
Economic Planning Agency	247	—	—	—	—	247
Science and Technology Agency	5,027	6,154	2,071	19,811	55,884	88,948
Environment Agency	2,309	425	—	—	—	2,734
Ministry of Justice	304	—	—	—	—	304
Ministry of Foreign Affairs	—	—	—	—	287	287
Ministry of Finance	131	—	—	—	—	131
Ministry of Education	3,238	11,096	30	—	—	14,365
Ministry of Health and Welfare	3,342	1,495	—	—	—	4,837
Ministry of Agriculture and Forestry	18,581	2,063	329	—	—	20,974
Ministry of International Trade and Industry	13,417	14,212	674	98	—	28,402
Ministry of Transportation	2,832	130	—	228	—	3,191
Ministry of Posts and Telecommunication	1,228	—	—	430	—	1,659
Ministry of Labour	211	—	—	—	—	211
Ministry of Construction	1,226	144	—	—	—	1,371
Ministry of Autonomy	179	—	—	—	—	179
Total	52,711	35,723	3,217	20,568	56,171	168,392

Expenditure in million yen.

agency submits its recommendations, based on the same guidelines it used to advise the research organizations. The allocation of funds is solely the prerogative of the Ministry of Finance.

There are about eighty institutes with a total staff of 16,000; many of them are associated with other ministries, and the agency holds discussions with all institutes. In the case of the Atomic Energy Bureau the agency recognizes its expertise and relies on it to coordinate all government research activities in the field of nuclear energy. The Research Coordinating Bureau carries out a similar function for space matters.

Special Funds

Although it may be said that gentle persuasion is the keynote of the agency's coordination, there are two devices by which projects may be positively promoted. The first is the provision of the Special Funds in the agency to promote research in priority areas or to accelerate research urgently required. Even though appropriation for the Funds still remains around 2% of the total expense for government research institutes in 1972, this device has been effective in enabling the agency to guide government research in a desired direction.

The second is the device for promoting so called "big science" or "big technology". As mentioned above, the development of nuclear energy was assigned to the agency from the outset and has grown up as a national project, consuming almost half the Expenses for Promotion budget for each year. Other similar projects are in space development and oceanographic development; coordination of research on the environment was, however, transferred to the Environment Agency when it started its work in 1971. In the fields of nuclear energy and space the coordination for

multidisciplinary approach for which the agency is well suited. Typical of such topics is life science which looks to be a promising area for the future.

Other Government Avenues

In this article I have chiefly examined the activities of the Science and Technology Agency but it must be noted that considerable efforts to promote science and technology are carried out by other organizations. For example, the Ministry of International Trade and Industry sponsors nine national projects at present whereas public corporations such as the Nippon Telegram and Telephone Corporation and the National Railway Corporation also give rise to substantial effects on the development of the technological capabilities of industry.

The Future

Generally speaking, I believe the Japanese experiment has been successful but the way ahead will not become easier for at least two reasons. First, the agency has brought nuclear energy and space development to a point at which they are self-sustaining and more areas will mature as time passes. As the number of such successes increases, so the task of overall coordination becomes more difficult. Second, the problems which face Japanese society in the 1970s are very different from those of the 1960s. As a consequence the agency must focus its attention on areas such as life science and the environment, but in so doing must inevitably extend government responsibility and involvement. The manner in which private industry may be encouraged and guided must also be worked out. The central machinery has proved itself capable of coping with the problems of the past decade, and building on its experience and success should prove equal to the challenge of the future.

Precambrian Africa and South America as a Single Continent

J. D. A. PIPER, J. C. BRIDEN & KEITH LOMAX

Department of Earth Sciences, University of Leeds

Palaeomagnetic evidence suggests that the principal cratonic areas of Africa were approximately in their present relative positions and orientations as early as 2,200 m.y. BP. This implies that the younger orogenic belts between them did not result from convergence of widely separated forelands which previously belonged to unrelated plates. South America and, until about 1,000 m.y. BP, North America may have been joined to Africa, and the concentration of all continental crust in one large mass until that time remains a possibility.

THE applicability of plate tectonics to pre-Mesozoic time is a topic of controversy and speculation. One view is that all orogenic belts mark the suturing of previously distinct crustal plates and form at the culmination of plate convergence^{1,2}. The opposite view is that some ancient orogenic belts developed between contiguous blocks of continental crust^{3,4}. Much of this discussion has been focused on Africa which contains the largest area of Precambrian crust, displaying a pattern of stable cratonic blocks and intervening orogenic belts ranging from older than 3,000 m.y. to Palaeozoic.

The argument is insoluble by geometrical plate tectonic analysis because the relevant Euler poles and rotations cannot be estimated^{5,6}, and discussion has consequently hinged on interpretation of stratigraphic and structural sequences. Principally the occurrence of features characteristic of modern convergent plate margins has been cited as evidence that African orogenic belts formed at plate margins⁵. On the other hand continuity of pre-orogenic features across younger orogenic belts has been regarded as evidence that orogeny was entirely ensialic^{4,7,8}. In particular within Africa, the continuity of the Buganda Toro-Kibalian and Ubendian Rusizian belts across the later Kibaran belt⁹, and the continuity of the Namaqualand (or Orange River) and Kibaran belts across the later Damaran-Lufilian belts with no apparent offset⁸, prohibit large scale subduction interpretations of these belts.

Other observations which have been taken to indicate that these and similar ancient orogenies were inherently different from more modern ones include the correlation of sediments deposited on the cratons with those within the orogenic zones⁸, the longer deformation history and less pronounced linearity of these belts compared with modern ones, the

higher percentage of granites within them⁴, and the differing metamorphic facies (and hence *P-T* conditions) which evidently pertained within them.

Other lines of evidence are less decisive and have been interpreted to favour either view. They include the problems of whether the reactivation of granitic basement is due to a thermal high in crust of no more than normal thickness, or to thickening of crust in paratectonic orogeny when two continental plates collide⁵; whether granulite facies basement within the belts is evidence of their *in situ* development⁸; and whether ⁸⁷Sr/⁸⁶Sr ratios 0.702 to 0.705 commonly measured in granitoid rocks throughout the belts decisively favour either model¹⁰.

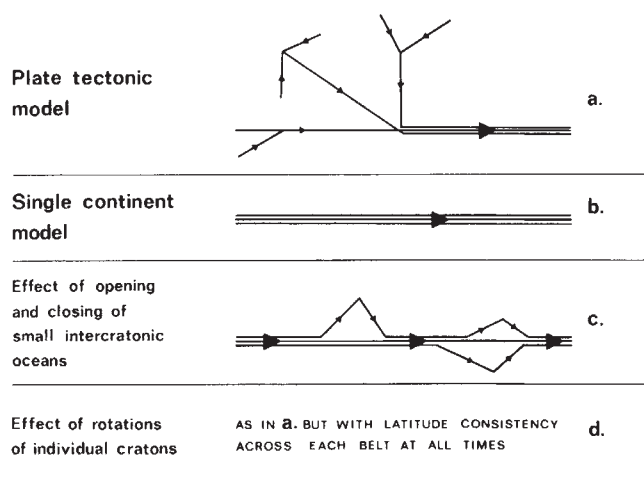


Fig. 1 Predicted pattern of palaeomagnetic polar wander paths.

It seems unlikely that the argument will ever be settled on geological grounds. It is our purpose here to point out that palaeomagnetic evidence is potentially decisive, and that a substantial amount of such data already exists and strongly favours the ensialic model for these orogenies rather than the plate tectonic model.

Palaeomagnetic Test

The two models predict completely different distributions of cratons prior to orogeny, and correspondingly different patterns of older palaeomagnetic poles. The plate tectonic model, if founded strictly on the Mesozoic to present analogy, requires that cratons bounding an orogenic belt should have been many thousands of kilometres apart only 100 to 200 m.y. before the fundamental deformation of the

belt. Thus prior to the 550 ± 100 m.y. "Pan-African" orogeny the West African, Congo and Kalahari cratons would have been far apart (at, say, 900 m.y.) and approaching one another. Going back one stage further the motions of the three cratons, themselves formed by coalescence of older and smaller nuclei, would have been completely different and as independent as are the motions of any set of modern plates. Each craton should display its own distinctive polar wander path up to the time of suturing with its neighbour (Fig. 1a). The "master" polar wander path could be thought of as applying to the Kaapvaal craton which is the most studied palaeomagnetically. Relative to it, other paths should form a dendritic pattern, converging to three principal stems by $1,100 \pm 200$ m.y. (the Kibaran-Namaqualand-Irumide orogeny) and to a single common path by the culmination of the Pan-African event.

The alternative single continent model, of ensialic orogeny, is more straightforward. The cratons or nuclei of pre-existing stable crust remained essentially stationary relative to each other while orogenic deformation developed between them, and all moved in concert relative to the poles. A single polar wander path is therefore predicted, applicable to the whole assemblage for the whole time the model applies (Fig. 1b).

In Fig. 1c and d, two possible relaxations from these two extreme models are illustrated. If the closure of an ocean were the precise negation of an earlier divergence, the common polar wander path would be punctuated by a detour marking the development and subsequent consumption of each intercratonic ocean (Fig. 1c). In our African example the three cratons would have been in the self-same relative positions at (say) 1,000 m.y. as they have been since the Pan-

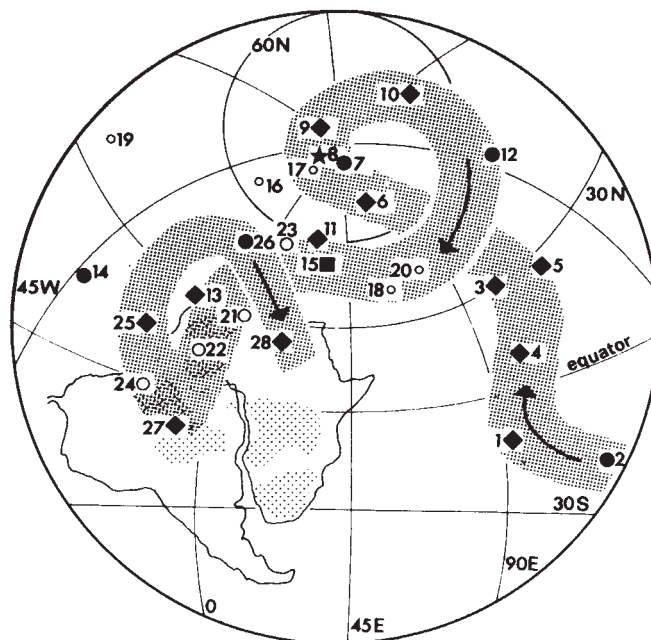


Fig. 3 Mean palaeomagnetic poles and polar wander path for African and South American rocks in the approximate age range 900 to 500 m.y. The presentation is similar to Fig. 2, and the data are numbered from Table 2. In this figure the cratonic areas stable since $1,100 \pm 200$ m.y. are indicated, and the origins of the palaeomagnetic data are denoted as follows: ●, South and South-west Africa; ◆, Central Africa; ★, Sudan; ■, West Africa; ○, South America. The apparent polar wander path is that of the south pole (see Fig. 4 and text).

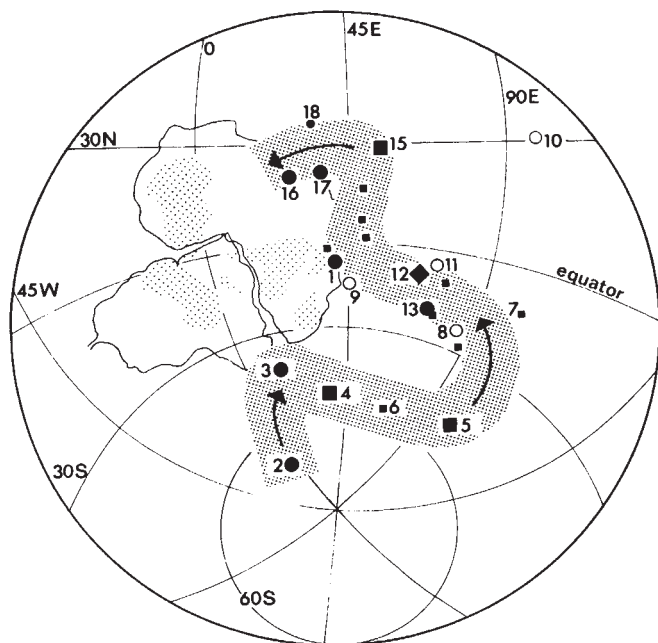


Fig. 2 Mean palaeomagnetic poles from African and South American rocks in the approximate age range 2,300 to 1,950 m.y. Africa is drawn in its present position, but South America is rotated, along with its palaeomagnetic poles, into the position computed by Bullard *et al.*¹⁴. Oblique stereographic projection. Cratonic areas which have been stable since at least 1,500 m.y. are stippled. The palaeomagnetic poles are numbered as in Table 1 (smaller symbols being used for data from single localities only; the small unnumbered squares refer to entry (14) in Table 1). The different cratons from which they were determined are distinguished as follows: ●, Kaapvaal; ◆, Congo; ■, West Africa; ○, Guyana. The polar wander path which is most consistent with these data is drawn as a finely-stippled swathe: this is the path of the north pole (see Fig. 4 and text).

African orogeny. The argument could be repeated for older cratons and the orogenic belts between them back to the oldest exposed situation exemplified by the granitic batholiths and intervening schist belts more than 3,000 m.y. old in the Rhodesia-Kaapvaal craton. This variant of the plate tectonic model presupposes *force majeure*, exercising long-term control on plate motions to a degree which is not readily discernible in the more recent analogy, but is mentioned because such controls seem to be implicit in the Palaeozoic plate-tectonic models of the North Atlantic region of Wilson¹¹, Dewey¹² and others, and may be tested in the context of Africa.

Finally, if substantial relative rotation of neighbouring cratons has occurred with little or no change in separation, the polar wander paths from the different cratons would appear as different as in Fig. 1a, but close inspection would show that inferred latitudes (but not orientations) were consistent across individual orogenic belts (Fig. 1d). An example of this class might be the Limpopo belt along which large scale lateral shear has been postulated¹³.

The identification of any one of the patterns of polar wander paths depicted in Fig. 1 is diagnostic of the corresponding continent distribution model unless Euler and palaeomagnetic poles have ever coincided. This is a restatement of the well known indeterminacy of longitude by palaeomagnetism, and the existence of long polar wander paths shows that this coincidence is rare; possible ambiguity only arises during "quasi-static intervals" when the pole seems to have remained in the same position for a long time.

Palaeomagnetic Evidence

Our chief purpose in this article is to present new palaeomagnetic data from Africa (Tables 1 and 2, Figs 2 and 3) which strongly favour the single continent model. It is appropriate to include South America in this discussion. In Figs 2 and 3 Africa retains its present outline and shape,

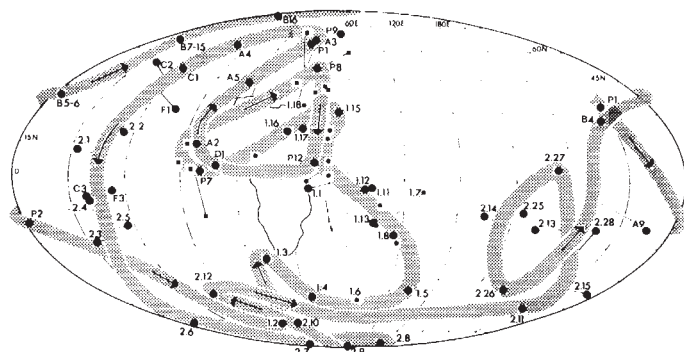


Fig. 4 Apparent north polar wander path relative to Africa, complete from ~2,700 m.y. to the present. Suffixes denote the various sources of data, as follows: 1 and 2, Tables 1 and 2 of this article; P and B, Tables 1 and 2 of ref. 15; A, ref. 18; C, ref. 17; D, ref. 47; F, ref. 37; small unnumbered dots refer to entry (14) of Table 1 of this article; small unnumbered squares refer to Waterberg sediments, ref. 48. These and other small symbols are poles derived from single localities only; all other poles are based on sampling at several localities and are denoted by large dots. The few poles which fall clear of the polar wander path (stippled swathe) are tied to the path at the appropriate point for their age. Mollweide projection; note the extreme distortion near the edges, which confuses the path near the south pole (see Figs 2 and 3). This seemingly tangled path is approximately 1,200 arc deg in length which, for a time-span of ~2,700 m.y., implies an average rate of apparent polar shift of ~0.4° per m.y. (~4 cm yr⁻¹).

and South America is placed adjacent to it in the position computed by Bullard *et al.*¹¹ which is supported for Palaeozoic and early Mesozoic times by palaeomagnetic and geological data.

In Fig. 2 we show that data in the approximate age range 2,300 to 1,900 m.y. from the Guyana, West African and Rhodesia-Kaapvaal cratons are, given their limitations and the uncertainty in ages, consistent with a single polar wander path. We note the close proximity and sequential positions of poles from the lavas of the Upper Ventersdorp and Transvaal Systems of South Africa (~2,300 to 2,200 m.y.) and poles from Ghana (~2,200 m.y.) and the West Africa and Guyana shields in general for the age range 2,200 to 2,100 m.y. The agreement of poles derived from rocks in the north-west Sahara associated with the Aftout Granite (~1,950 m.y.) and from rocks of indistinguishably different age from South Africa is equally impressive. We conclude that the "base map" of Fig. 2 is correct.

The same conclusion is drawn from Fig. 3 which shows a similar sequence of palaeomagnetic poles for the approximate age range 900 to 500 m.y. derived from Argentina, the Sudan, Tanzania, Zambia, Malawi, South and South-west Africa. There is close agreement of poles ascribed to similar ages from rocks as widely separated as the Sudan and South Africa, and the large early Cambrian polar shift is established independently from Zambia, south-western Africa and Argentina.

Age estimates are not everywhere consistent with the simple common polar wander paths implied in Figs 2 and 3, but it may be that the assigned ages are wrong rather than the palaeomagnetic interpretation. The general agreement of the data with the single continent model is most striking, and evidently disagrees strongly with the plate tectonic model although small amounts of subsequently compensated relative rotations and separations cannot be ruled out.

The foregoing discussion shows that it may be correct to treat Africa as a single continental plate for palaeomagnetic purposes from 2,300 m.y. onwards, and to compile a single

Table 1 Data for Approximately 2,300 to 1,950 m.y.

No.	Rock unit/source area	Ref.	Age (m.y.)	Mean palaeomagnetic pole*	Reliability†
1	Lower Ventersdorp lavas, South Africa	31	~2,300‡	4° S 40° E	N, a, (t), k, f
2	Upper Ventersdorp lavas, South Africa	31	2,300±100	71° S 353° E	N, a, t, k
3	Lavas, Transvaal System, South Africa	J.C.B. unpublished	~2,250	42° S 14° E	N, a, f, k
4	Tarkwaian intrusions, Ghana	32	~2,200	53° S 36° E	N, a, f, k
5	Obuasi greenstone body, Ghana	32	2,200§	50° S 102° E	N, k
6	Dolerite dyke, Obuasi, Ghana	32	<2,200	56° S 68° E	n, a
7	Dolerite intrusion, Ivory Coast	32	<2,200	11° S 102° E	n, a
8	Roraima dolerites, Guyana (i)	33	1,500-2,090	(63° S 51° E) 23° S 86° E	N, a, k
9	Roraima dolerites, Guyana (ii)	33	1,500-2,090	(45° S 347° E) 14° S 45° E	N, a, k
10	Blackawatra dykes, Suriname	34	? 1,550-1,650	(8° S 53° E) 31° N 97° E	n, a, t, k
11	Kabaledo dolerites, Suriname	34	? ~1,750	(44° S 30° E) 4° S 74° E	N, a, t, k
12	Angola anorthosite, Angola	J. D. A. P., unpublished	¶	9° S 69° E	N, a, k
13	Orange River lavas, South Africa	J. D. A. P., unpublished	>1,850	19° S 74° E	N, a, k
14	Aftout diorite and associated dolerite dyke, Algeria	K. L., unpublished	~1,950	Poles from seven sites illustrated in Fig. 1	N, a, (k large at each site)
15	Aftout gabbro, Algeria	K. L., unpublished	~1,950	29° N 55° E	N, a, k
16	Basic granophyre, Vredefort Ring Complex, South Africa	35	~1,970	22° N 27° E	N, a, k
17	Bushveld Complex, South Africa	36	1,950±50	23° N 36° E	N, f, k
18	Losberg intrusion, South Africa	35	~1,950	33° N 36° E	a, k

* Pole positions relative to present coordinates of Africa, South American poles being rotated to allow for closure of the South Atlantic. Present coordinates of South American poles are given in brackets.

† All data are statistically significant, but additional evidence for reliability is indicated as follows (in general the more entries the better): N(n), more than 20 (10) samples; a, alternating field demagnetization; t, thermal demagnetization; f, fold test; k, Fisher precision greater than 10.

‡ Magnetization may date from metasomatism at about 2,150 m.y.

§ Magnetization may date from metasomatism at about 2,200 m.y.

|| Most probable age about 2,070 m.y.

¶ Age determinations range from 1,300 to 2,600 m.y.

** Some later data are mentioned in ref. 41.

polar wander path applicable to the whole continent throughout that time. The data summarized here, when added to those already published¹⁵⁻¹⁸, provide a sufficiently complete record to eliminate the ambiguity in "polarity" which has hitherto made it impossible to determine whether particular isolated segments of the path were the track of the north or south pole. By continuity, it now emerges that the path for 2,300 to 1,900 m.y. in Fig. 2 is the track of the north pole, but that the path for 900 to 500 m.y. illustrated in Fig. 3 is that of the south pole. The complete north polar wander path from 2,300 m.y. to the present is drawn in Fig. 4, in which data from 2,700 to 2,300 m.y. from Rhodesia and the Transvaal are added in spite of the absence of corroborative data from other cratons for that interval. The paths for the intervals 2,700 to 2,300 m.y. and 1,950 to 1,300 m.y., which were the only parts which could be estimated in 1968 (ref. 15), are now the least well defined portions of the whole path. Indeed it is no longer certain that the loop drawn for that latter interval by the 1968 authors is real; several of the poles formerly regarded as ~1,700 m.y. old which helped to define that loop are now believed to be younger, 1,200 to 1,000 m.y.

Supercontinent before 1,000 m.y.?

The data of Fig. 5 show that the polar wander paths for Africa and North America can be rotated into fair agree-

ment for the time interval 2,700 to 1,000 m.y. and that when this is done the two continents rotate into adjacent positions so that they could have belonged to a single plate¹⁸. Continuity with Phanerozoic data indicates that these paths are the north polar wander paths for each continent^{18,19}. Their mutual divergence after 1,000 m.y. implies that they then moved towards their Lower Palaeozoic relative positions and that at least one active plate margin became operative between them. Evidence for the existence of this supercontinent before 1,000 m.y., incorporating North America, Africa and, from our foregoing discussion, South America, is strongest for the interval 1,400 to 1,000 m.y. (ref. 18). Although the older evidence is less convincing the overall similarities between the North American and African polar wander paths back beyond 2,000 m.y. are more striking than their differences—the more so if the starting point of the North American path at 2,700 m.y. is plotted as shown in Fig. 5 rather than by taking the antipodal starting point^{19,20}. Admittedly the ages attached to corresponding points on the two paths do not all seem to agree, and there are small scale separations and convergences suggestive of the scheme shown in Fig. 1c, but the reality of these complications is unproven.

In his more detailed discussion of the period, 1,400 to 1,000 m.y., J. D. A. P. has shown that, when these portions of the North American and African polar wander paths are superimposed, the Grenville, Kibaran and Namaqualand orogenic

Table 2 Data for Approximately 900 to 500 m.y.

No.	Rock unit/source area	Ref.	Age (m.y.)	Mean palaeomagnetic pole*	Reliability†
1	Kigonero Flags, Tanzania	37	> 890	12° S 93° E	N, t, k
2	Dykes, Klein Karas, South-west Africa	18	878 ± 41	20° S 114° E	n, a
3	Gagwe amygdaloidal lavas, Tanzania	37	813 ± 30	29° N 103° E	N, a
4	Bukoban intrusives, Tanzania	37	806 ± 30	11° N 101° E	N, a
5	Manyovu redbeds, Tanzania	37	< 806 ± 30	24° N 118° E	N, t
6	Mbozi complex, Tanzania	17	743 ± 30	72° N 68° E	N, a
7	Pre-Nama dykes, South-west Africa	18	653 ± 70	85° N 228° E	N, a, k
8	Sabaloka ring structure, Sudan	38	> 540	83° N 339° E	n, a, f, k
9	Lower Buanji Series, Tanzania	J. D. A. P., unpublished	< 1,350	87° N 263° E	N, a, t, k
10	Plateau Series, Zambia (i)	17	< 1,000	71° N 173° E	N, a, k
11	Plateau Series, Zambia (ii)	17	< 1,000	60° N 25° E	n, a, k
12	Fish River Series, South-west Africa	18	? Lower Cambrian	55° N 137° E	n, a
13	Ntonya Ring Structure, Malawi	39	630 ± 24	28° N 345° E	N, a, t, k
14	Klipheuvelformation, South Africa	40	? Lower Cambrian	16° N 316° E	n, otherwise not known
15	Lavas, Morocco	42	"Middle Cambrian"	53° N 34° E	a, otherwise not known
16	Sediments, South Tilcara, Argentina	43	"Cambrian"	(52° N 27° E) 66° N 330° E	n, t
17	Sediments, North Tilcara, Argentina	43	"Cambrian"	(49° N 24° E) 80° N 350° E	n, t
18	Sediments, Abra de Cajas, Argentina	43	"Cambrian"	(2° N 28° E) 41° N 66° E	t
19	Sediments, Purmamarca village, Argentina	43	"Cambrian"	(61° N 293° E) 36° N 295° E	t
20	Sediments, Purmamarca, Argentina	43	"Cambrian"	(5° N 39° E) 45° N 80° E	t, k
21	Red sediments, Salta and Jujuy, Argentina	44	Cambro-Ordovician	(15° N 338° E) 30° N 3° E	n, t
22	Red sediments, Salta and Jujuy, Argentina	44	Cambro-Ordovician	(8° N 320° E) 13° N 353° E	n, t
23	Sediments, Salta, Argentina	43	Ordovician	(31° N 13° E) 56° N 9° E	n, t
24	Sediments, Bolivia	44	Ordovician	(4° N 302° E) 1° S 342° E	n, t, f
25	Hook intrusives, Zambia	45	500 ± 17	14° N 336° E	n, a, k
26	Table Mountain Series, South Africa	46	Ordovician	50° N 349° E**	a, k
27	Plateau Series, Zambia (iii)	17	Lower Palaeozoic	10° S 352° E	a, k
28	Plateau Series, Zambia (iv)	17	Lower Palaeozoic	22° N 19° E	n, k

Footnotes as Table 1.

belts lie on a single large arc. This palaeomagnetic evidence suggests that the African portions of this arc were the site of ensialic orogeny, while according to Irving *et al.*²¹ the North American portion was the site of plate convergence. If these interpretations are correct then the two facets of orogeny were occurring simultaneously alongside each other around 1,300 to 1,000 m.y. shortly before this Precambrian supercontinent began to break up. Perhaps the Grenville "suture" was the precursor of the plate margin(s) the activity of which caused this breakup. If so, an understanding of late Proterozoic plate kinematics might be gained from studying how the

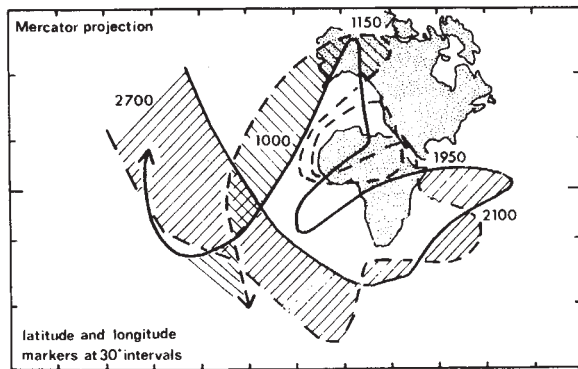


Fig. 5 Schematic apparent polar wander paths from ~2,700 to ~900 m.y. for Africa (see Fig. 4) and North America (after Irving and Park¹⁹ modified near 2,700 m.y. as described in the text). Approximate ages of selected parts of the curve are marked in m.y. The discrepancies between possibly corresponding parts of each curve are shaded to the left for 2,700 to 1,950 m.y., and to the right for 1,150 to 900 m.y.; in the intervening period no shading has been inserted because of the possible complications in the African curve. A curiosity of this map is that in making this approximate match of the two curves, both continents retain their present latitude and orientation; this is of dubious geophysical significance and it is beyond the scope of this article to speculate on it.

1,000 m.y. reassembly (Fig. 5) could have evolved into postulated reassemblies for early Palaeozoic time^{6,22}.

Precambrian "Pangaea"?

It has previously been pointed out that the bulk of Precambrian palaeomagnetic data from Australia^{23,24} and India²⁴ are consistent with their having remained in positions relative to Africa as in Carey's²⁵ Gondwana reassembly. On the other hand Phanerozoic data are more consistent with a different Gondwana reassembly^{26,27}. Late Proterozoic data²⁸ seem to fit neither of these and it is not clear whether they are compatible with transition from the one to the other. An assessment of these problems in the light of new Australian data is currently being made by M. W. McElhinny and colleagues (personal communication).

Finally, the available Precambrian data from Europe²⁹ do not show the loops which are typical of North American and African polar wander paths, but this could reflect absence of data for critical periods. We note that European and North American Precambrian poles lie in the same general regions after allowance for Phanerozoic opening of the North Atlantic.

In summary, the palaeomagnetic evidence that Africa and South America were parts of a single continent in Precambrian times is rather compelling. The evidence that North America belonged to this same continent until ~1,000 m.y. is merely persuasive. That other parts of surviving continental crust also belonged is only conjecture which is not contradicted by the evidence. Conclusive solutions to the outstanding problems will not easily be reached for many reasons: the geological record is far from ideal; it is difficult to prove identity or the significance of differences between

palaeomagnetic data; and congruence of segments of polar wander paths is not in itself proof that they should be superimposed over their whole length. But the eventual outcome will affect our conclusions only by degree. This demonstration that some Precambrian orogenic belts within Africa formed between cratons which had already been neighbours for hundreds of millions of years is sufficient to establish the need for a supplement to plate theory of orogenesis. J. C. B. speculated briefly on this matter elsewhere³⁰.

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- ¹ Dewey, J. F., and Horsfield, B., *Nature*, **225**, 521 (1970).
- ² Dickinson, W. R., *Science*, N.Y., **174**, 107 (1971).
- ³ Clifford, T. N., in *African Magmatism and Tectonics* (edit. by Clifford, T. N., and Gass, I. G.) (Oliver and Boyd, Edinburgh, 1970).
- ⁴ Shackleton, R. M., in *Time and Place in Orogeny* (edit. by Kent, P. E., *et al.*), *Geol. Soc. Lond., Spec. Publ.*, **3**, 1 (1969).
- ⁵ Burke, K., and Dewey, J. F., in *African Geology* (edit. by Dessauvage, T. F. J., and Whiteman, A. J.), 583 (University of Ibadan, 1970).
- ⁶ Smith, A. G., Briden, J. C., and Drewry, G. E., in *Organisms and Continents through Time* (edit. by Hughes, N. F.), *Spec. Pap. Palaeont.*, **12**, 1 (1973).
- ⁷ Anheuser, C. R., Mason, R., Viljoen, M. J., and Viljoen, R. P., *Bull. geol. Soc. Am.*, **80**, 2173 (1969).
- ⁸ Clifford, T. N., in *Radiometric Dating for Geologists* (edit. by Hamilton, E. I., and Farquhar, R. M.), 299 (Interscience, New York, 1968).
- ⁹ Cahen, L., in *African Magmatism and Tectonics* (edit. by Clifford, T. N., and Gass, I. G.), 97 (Oliver and Boyd, Edinburgh, 1970).
- ¹⁰ Hurley, P. M., *Earth planet. Sci. Lett.*, **15**, 305 (1972).
- ¹¹ Wilson, J. T., *Nature*, **211**, 676 (1966).
- ¹² Dewey, J. F., *Nature*, **222**, 124 (1969).
- ¹³ Coward, M. P., Graham, R. H., James, P. R., and Wakefield, J., *Sixteenth A. Rep. Res. Inst. afr. Geol.* (University of Leeds, 1972).
- ¹⁴ Bullard, E. C., Everett, J. E., and Smith, A. G., *Phil. Trans. R. Soc. Lond.*, **A258**, 41 (1965).
- ¹⁵ McElhinny, M. W., Briden, J. C., Jones, D. L., and Brock, A., *Rev. Geophys.*, **6**, 201 (1968).
- ¹⁶ Brock, A., and Piper, J. D. A., *Geophys. J.*, **28**, 139 (1972).
- ¹⁷ Piper, J. D. A., *Geophys. J.* (in the press).
- ¹⁸ Piper, J. D. A., *Geophys. J.* (in the press).
- ¹⁹ Irving, E., and Park, J. K., *Can. J. Earth Sci.*, **9**, 1318 (1972).
- ²⁰ Spall, H. R., *Earth planet. Sci. Lett.*, **10**, 273 (1971).
- ²¹ Irving, E., Park, J. K., and Roy, J. L., *Nature*, **236**, 344 (1972).
- ²² Briden, J. C., Morris, W. A., and Piper, J. D. A., *Geophys. J.* (in the press).
- ²³ Porath, H., *Earth planet. Sci. Lett.*, **2**, 409 (1967).
- ²⁴ Piper, J. D. A., in *Implications of Continental Drift to the Earth Sciences* (edit. by Tarling, D. H., and Runcorn, S. K.), 1, 19 (Academic Press, London, 1973).
- ²⁵ Carey, S. W., in *Continental Drift—A Symposium* (edit. by Carey, S. W.), 177 (Univ. Tasmania, Hobart, 1956).
- ²⁶ Smith, A. G., and Hallam, A., *Nature*, **225**, 139 (1970).
- ²⁷ McElhinny, M. W., and Luck, G. R., *Science*, N.Y., **168**, 830 (1970).
- ²⁸ Embleton, B. J. J., *Earth planet. Sci. Lett.*, **17**, 217 (1972).
- ²⁹ Spall, H. R., *Earth planet. Sci. Lett.*, **18**, 1 (1973).
- ³⁰ Briden, J. C., *Nature*, **244**, 400 (1973).
- ³¹ Henthorn, D. I., thesis, Univ. Leeds (1972).
- ³² Piper, J. D. A., and Lomax, K., *Geophys. J.* (in the press).
- ³³ Hargraves, R. B., *Geophys. J.*, **16**, 147 (1968).
- ³⁴ Veldkamp, J., Mulder, F. G., and Zijdeveld, J. D. A., *Phys. Earth planet. Int.*, **4**, 370 (1971).
- ³⁵ Hargraves, R. B., *J. Geol.*, **78**, 253 (1970).
- ³⁶ Gough, D. I., and van Niekerk, C. B., *Phil. Mag.*, **4**, 126 (1959).
- ³⁷ Piper, J. D. A., *Geophys. J.*, **28**, 111 (1972).
- ³⁸ Briden, J. C., *Seventeenth Rep. Res. Inst. afr. Geol.* (University of Leeds, in the press).
- ³⁹ Briden, J. C., *J. geophys. Res.*, **73**, 725 (1968).
- ⁴⁰ Creer, K. M., and Snape, C., quoted in ref. 41.
- ⁴¹ Creer, K. M., in *Implications of Continental Drift to the Earth Sciences* (edit. by Tarling, D. H., and Runcorn, S. K.), 1, 47 (Academic Press, London, 1973).
- ⁴² Helsley, C. E., *Trans. Am. geophys. Un.*, **46**, 67 (1965).
- ⁴³ Thompson, R., *Earth planet. Sci. Lett.*, **18**, 266 (1973).
- ⁴⁴ Creer, K. M., *Phil. Trans. R. Soc. Lond.*, **A267**, 457 (1970).
- ⁴⁵ Brock, A., *Nature*, **216**, 359 (1967).
- ⁴⁶ Graham, K. W. T., and Hales, A. L., *Geophys. J.*, **5**, 318 (1961).
- ⁴⁷ Brock, A., Raja, P. K. S., and Vise, J. B., *Geophys. J.*, **28**, 129 (1972).
- ⁴⁸ Jones, D. L., and McElhinny, M. W., *J. geophys. Res.*, **72**, 4171 (1967).

Calcium Ionophores and Movement of Calcium Ions following the Physiological Stimulus to a Secretory Process

J. C. FOREMAN & J. L. MONGAR

Department of Pharmacology, University College London, London WC1E 6BT

B. D. GOMPERTS

Department of Experimental Pathology, University College Hospital Medical School, London WC1E 6JJ

Mast cells can be induced to secrete histamine by an ionophore, the action of which depends on the presence of calcium within a specific concentration range. The fact that labelled calcium can be seen to enter sensitized cells during histamine secretion in the presence of antigen suggests that the entry of calcium into the mast cell may be the trigger for the release of histamine.

CHANGES in the intracellular concentration of calcium ions provide the control for many physiological processes, including secretion¹. The isolated mast cell is a useful model for studying the mechanism of secretion because the degree of secretory activity is readily assessed by assay of the histamine that is released, and the external environment of the free cells can be rapidly changed and controlled. The secretion of histamine from mast cells stimulated by the antigen-antibody reaction is dependent on calcium^{2,3} and it has been suggested that histamine secretion is the result of the influx of calcium into the mast cell, following a change in the calcium permeability of the cell membrane induced by the antigen-antibody reaction^{4,5}.

Ionophores⁶ which selectively increase the permeability of cell membranes to calcium would be valuable tools for the investigation of physiological processes. Two ionophores, X537A (Hoffmann-La Roche) and A23187 (Eli Lilly), transport calcium across sarcoplasmic reticulum^{7,8}, but Pressman has pointed out⁹ that X537A is not a specific carrier for divalent cations and will transport monovalent cations and some primary amines. We have studied the effects of A23187 and X537A on isolated rat mast cells.

The ionophores are insoluble in aqueous media and were added to the cells as a suspension in Tyrode solution, which has the following composition (mmol l⁻¹): NaCl, 137; KCl, 2.7; NaH₂PO₄, 0.4; NaHCO₃, 12; glucose, 5.6; calcium and magnesium chlorides were added as required. For most of the experiments, mixed peritoneal cells of rat were used⁸. Partial fractionation of the peritoneal cells was performed in some experiments by density gradient centrifugation in bovine serum albumin¹⁰. Histamine release was measured by incubating aliquots of cells with the ionophore for 10 min at 37° C, after which the cells were diluted with ice-cold Tyrode solution and centrifuged. Histamine in the supernatant and that remaining in the pellet was assayed biologi-

cally. Histamine release is expressed as a percentage of total and is corrected for release occurring in the absence of ionophore (about 2%). The assay was unaffected by the ionophore at the dilutions encountered.

Calcium movement in peritoneal cells was estimated by measuring changes in the absorbance of murexide in a dual wavelength spectrophotometer. The cells were suspended in magnesium-free Tyrode solution in which the bicarbonate and phosphate had been replaced by HEPES buffer (10 mmol l⁻¹, pH 7.6) and the calcium concentration was 1 mmol l⁻¹. The other technique for monitoring calcium movement was based on a method described by Harris and Berent¹¹. Cells were incubated in Tyrode solution containing calcium, 1 mmol l⁻¹ at 37° C, and at zero time a small quantity of radioactive calcium (⁴⁵Ca) in the same Tyrode solution was added, together with antigen if required. After a predetermined time, the cells were separated from the radioactive loading solution by centrifuging them at about 12,000g through a water-impermeable barrier of silicone oil ('Versilube F 50') into urea, 6 mol l⁻¹. The ⁴⁵Ca in the urea layer and in the loading Tyrode solution was estimated by liquid scintillation spectroscopy. Correction for ⁴⁵Ca carried through the silicone layer in extracellular water was made by estimating the entrained water space with either ¹⁴C-hydroxymethyl inulin or ³⁵SO₄: both compounds gave similar corrections.

A23187 and X537A caused release of histamine from mast cells. A23187 was active in the concentration range 0.1 to 1.0 μmol l⁻¹ but X537A was active in a concentration range about an order of magnitude greater (1–10 μmol l⁻¹). Both compounds produced very steep concentration-effect relationships: a two-fold change in concentration almost covered the entire range of response. Measurements of the fluorescence of A23187 have shown that the cells extract the ionophore from the aqueous suspending medium, and so the concentrations given are those before the addition of cells, and the amount of ionophore within the cell membrane will depend on the concentration of cells as well as on the initial concentration of the ionophore.

Comparison of Calcium and Strontium

Calcium alone does not induce secretion of histamine from mast cells. In the absence of calcium, A23187 caused only slight release of histamine, which increased on addition of calcium to the extracellular medium (Fig. 1). The ionophore also induced uptake of calcium by rat peritoneal cells (Fig. 2).

In contrast, the action of X537A was totally independent of calcium and released between 80 and 90% of the histamine from cells in a medium free of calcium. The range of

concentration over which the calcium is active in the presence of A23187 is similar to the range of concentration over which it is active in antigen-antibody-induced histamine secretion⁹, suggesting the possibility of a common receptor site for calcium in both instances.

Strontium also activates the release of histamine in the presence of A23187 (Fig. 1) over the same range of concentration in which it activates antigen-antibody-induced histamine secretion. The histamine secretion in the presence of strontium seems to be small compared with that in the presence of calcium, but these histamine releases were measured after only 10 min of incubation and further experiments have shown that the release of histamine in the presence of strontium and the ionophore continues to increase with time. It thus seems that A23187 transports enough calcium to activate the release process more rapidly than it transports the commensurate amount of strontium.

Effect of Metabolic Inhibitors

Release of histamine by calcium and A23187 cannot proceed when glycolysis and mitochondrial respiration are inhibited (Fig. 3). X537A, on the other hand, is effective even when glycolysis and mitochondrial respiration have been inhibited and independently of the presence of calcium, which suggests that it may act by a completely different mechanism. One possibility is the simple exchange of extracellular sodium ions for histamine within the intracellular storage granules without exocytosis, which is normally thought to occur after the fusion of cell and granule membranes and the consequent extrusion of membrane-free granules from the cytoplasm¹². In contrast, A23187 seems to act by transporting calcium into the cell and thereby initiating a reaction, dependent on intact metabolic processes, which results in the secretion of histamine.

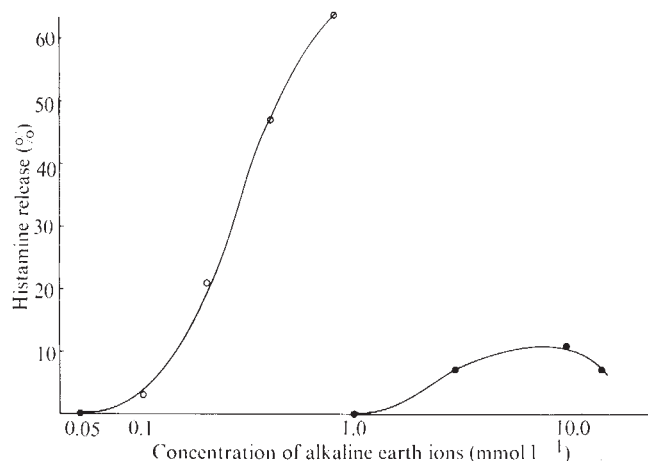


Fig. 1 Concentration-effect curves for calcium and strontium in the presence of A23187, $0.6 \mu\text{mol l}^{-1}$, on histamine secretion from rat mast cells. $\circ$ — $\circ$, Calcium; $\bullet$ — $\bullet$, strontium.

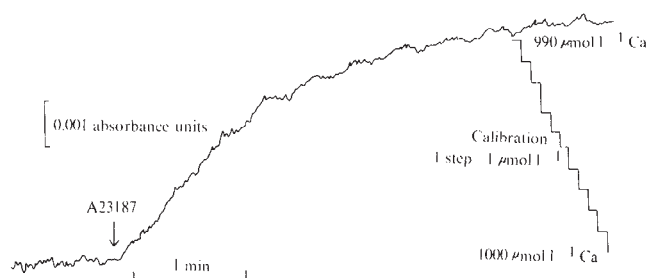


Fig. 2 Change in absorbance of murexide, $80 \mu\text{mol l}^{-1}$, induced by A23187, $3 \mu\text{mol l}^{-1}$, in the presence of a partially purified mast cell suspension. A23187 had no effect in the absence of cells and histamine, in concentrations likely to be released, had no effect. Murexide did not cause histamine release from the mast cells. Absorbance measured in a dual wavelength spectrophotometer at 470 nm, reference beam 510 nm.

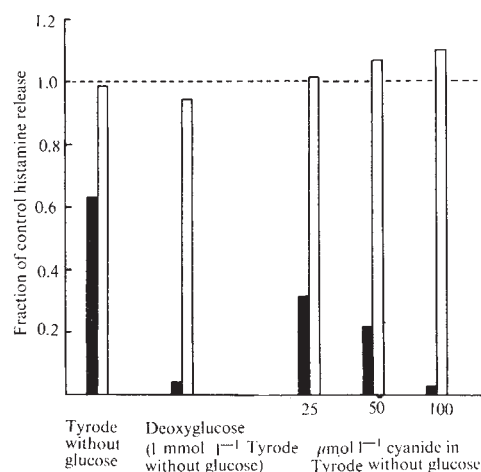


Fig. 3 The effect of inhibiting glycolysis and mitochondrial respiration on histamine secretion induced by A23187, $0.6 \mu\text{mol l}^{-1}$ ($\blacksquare$), and X537A, $17 \mu\text{mol l}^{-1}$ ($\square$), in the presence of 1.8 mmol l^{-1} calcium. Ordinate is the fraction of the control histamine releases in Tyrode solution which were: A23187—64%; X537A—83%. Cells were preincubated with inhibitor for 30 min before the addition of ionophore.

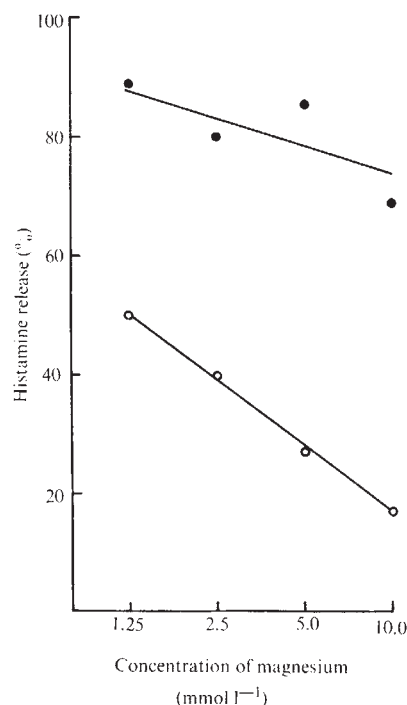


Fig. 4 The effect of magnesium on the histamine secretion induced by calcium in the presence of A23187, $1.2 \mu\text{mol l}^{-1}$. $\circ$ — $\circ$, 0.5 mmol l^{-1} calcium; $\bullet$ — $\bullet$, 2.0 mmol l^{-1} calcium.

If A23187 transports calcium into the mast cell, it is likely that some other species is carried in the opposite direction, for the ionophore is thought to act as a carrier. The transport of this species out of the cell may initiate the secretory process. Magnesium is one possible candidate for exchange with calcium. At an extracellular magnesium concentration of 10 mmol l^{-1} , the response to calcium in the presence of the ionophore is depressed (Fig. 4) but this depression is overcome by raising the calcium concentration. Because the extracellular concentration of 10 mmol l^{-1} is substantially higher than the normal intracellular magnesium concentration, it is unlikely that magnesium is transported from the cell down a concentration gradient. It is still possible, however, to elicit secretion of histamine with calcium in the presence of the ionophore. Thus it seems that the transport of magnesium out of the cell, in exchange for

Table 1 Action of Antigen on ^{45}Ca Movement in Sensitized and Non-sensitized Rat Peritoneal Cells

Experiment	Incubation time (min)	Total histamine per sample (ng)	Histamine release (%)	⁴⁵ Ca in cells (c.p.m.)*		Number of observations	Change due to antigen (c.p.m.)	⁴⁵ Ca in load solution (c.p.m. × 10 ⁶)
				No antigen	Antigen			
Sensitized								
1	1	180	40	2,511 ± 106	4,065 ± 274	5	1,553	1.0
2	1	100	40	2,948 ± 90	7,624 ± 533	5	4,676	1.2
3	1	350	24	5,042 ± 1,190	8,139 ± 585	3	3,097	1.9
4	1	400	26	5,672 ± 2,180	8,058 ± 1,710	3	2,386	2.7
5	2	700	25	3,120 ± 1,303	4,350 ± 1,113	3	1,230	1.0
Non-sensitized								
1	1	700	0	4,520 ± 257	4,693 ± 126	3	173	1.3

* Mean $\pm$ s.e. Cells suspended in a medium containing calcium, 1 mmol l^{-1} at 37°C .

calcium, is not essential for histamine secretion. The reduction in the response to calcium caused by magnesium may be due to competition between the two ions, similar to that described for antigen-antibody-induced secretion of histamine³.

The Physiological Stimulus and Calcium Movement

The experiments with A23187 suggest that entry of calcium into the mast cell is a sufficient stimulus to the metabolically-dependent mechanism which results in histamine secretion. One possible way in which the antigen-antibody reaction might induce histamine secretion would be by allowing the entry of calcium into the mast cell. Our preliminary observations on the movement of ^{45}Ca suggest that this hypothesis is reasonable. Table 1 shows that when sensitized mast cells with membrane bound antibody are stimulated with antigen in the presence of radioactive calcium, there is an increase in the amount of label associated with the cells. At 37°C this movement of ^{45}Ca stimulated by antigen is almost complete in the first fifteen seconds following stimulation, which corresponds to the rate of histamine release. The technique does not allow a distinction between uptake of calcium or exchange of the label with non-labelled calcium. The effect of antigen does

not seem to be a non-specific effect of the antigen protein causing binding of ^{45}Ca to the cell surface because it is not observed with non-sensitized cells having no fixed antibody.

Our experiments suggest that calcium entry into mast cells is the trigger to the secretory response.

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- Rubin, R. P., *Pharmac. Rev.*, **22**, 389 (1970).
- Mongar, J. L., and Schild, H. O., *J. Physiol.*, **140**, 272 (1958).
- Foreman, J. C., and Mongar, J. L., *J. Physiol.*, **224**, 753 (1972).
- Allison, A. C., *Chem. Phys. Lipids*, **8**, 374 (1972).
- Orr, T. S. C., and Allison, A. C., *Nature*, **236**, 350 (1972).
- Pressman, B. C., Harris, E. J., Jagger, W. S., and Johnson, J. H., *Proc. natn. Acad. Sci. U.S.A.*, **58**, 1949 (1968).
- Caswell, A. H., and Pressman, B. C., *Biochem. biophys. Res. Commun.*, **49**, 292 (1972).
- Scarpa, A., Baldassare, J., and Inesi, G., *J. Gen. Physiol.*, **60**, 735 (1972).
- Pressman, B. C., in *The Role of Membranes in Metabolic Regulation* (edit. by Mehlman, M. A., and Hanson, R. W.), 149 (Academic Press, New York, 1972).
- Perera, B. A. V., and Mongar, J. L., *Immunology*, **6**, 472 (1963).
- Harris, E. J., and Berent, C., *Biochem. J.*, **115**, 645 (1969).
- Thon, I. L., and Unvas, B., *Acta physiol. scand.*, **71**, 303 (1967).

LETTERS TO NATURE

PHYSICAL SCIENCES

Lakes Beneath the Antarctic Ice Sheet

THE technique of radio-echo sounding of polar ice sheets is now well established¹⁻³, and three seasons of radio-echo sounding from long range aircraft of the US Navy have been completed under a joint programme of the Scott Polar Research Institute and the US National Science Foundation.

During the most recent field season (1971-72) the square network of flight lines shown in Fig. 1 was flown over East Antarctica using SPRI Mark IV equipment operating at 60 MHz. The map also includes some flight lines from the 1969-70 season at longitudes beyond 90°E which are relevant to our discussion. During the 1971-72 season the improved radio-echo and navigational equipment gave excellent results

over most of the flight lines. A detailed survey of the bedrock echo characteristics over this area has been made during the past year. We conclude that this evidence, in combination with other studies, points to the existence of a number of water pockets or lakes a few kilometres wide under certain parts of the ice sheet.

Our film recording system produces a profile of ice thickness similar to that of marine echo sounding (Fig. 2a-d). From an aircraft flying at constant altitude profiles of both top and bottom surfaces are recorded as well as reflexions from within the ice mass. Although the strong echo from the upper surface of the ice shows little variation in strength, the normal bottom echo from inland ice (Fig. 2a) shows strong fading along the flight line. The horizontal correlation distance or "fading length" of the echo pattern along the flight path is of the order of 20 m and the fading range (variation of echo strength) approaches $\pm 10 \text{ dB}$ about the mean value. Harrison⁴ has

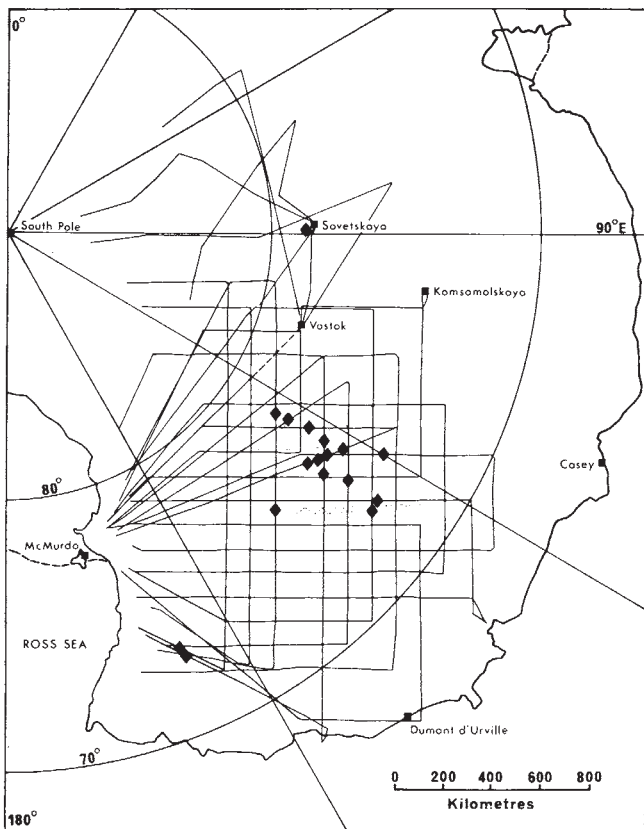


Fig. 1 Flight lines completed in the 1971-72 season of SPR-NSF radio-echo sounding programme. Some lines from 1969-70 are also included. ♦, Positions of the sub-ice lakes observed on these flights; ■, scientific stations.

shown that these characteristics can be explained in terms of the statistical properties of the ice-rock and ice-air surfaces. To produce fading as seen in Fig. 2 requires a bedrock roughness with a horizontal scale of 20 m or less and a vertical scale comparable to the wavelength of the radio waves (3 m in ice). We note also that the irregular echo returns persist for two or three microseconds after the first arrival, as a result of the broad beamed antenna used for sounding which collects scattered energy returns through angles up to 35° from the vertical. The echo pattern could result either from an irregular distribution of morainal rocks of sufficient size in the basal ice, or from the bedrock roughness mentioned above.

We have noted a number of sites, shown in Table 1, where the normal fading characteristics of the bottom echo change to that which we would expect from an extended smooth surface. Examples are shown in Fig. 2*b, c* and *d*. At these

sites, the fading length increases to 200 m and more, fading of the echo is much reduced and is absent in some cases, as seen at an enlarged horizontal scale in Fig. 2*d*. At the same time the duration of the bottom echo decreases to a length similar to that of the transmitted pulse (1.0 μ s for Fig. 2*b, c* and *d*). These characteristics all indicate that specular reflexion is taking place from an extended smooth surface. These surfaces seem to be almost horizontal, with slopes generally less than 1 in 150, except for one case with a slope around 1 in 50.

There is a marked increase in echo strength of 10 to 20 dB as we move from an area of normal fading to one of specular reflexion. The reflexion coefficient of an ice-rock interface is estimated to range from -14 to -20 dB (ref. 2), whereas the reflexion coefficient at an ice-pure water interface would be around -3 dB, provided we are dealing with a thickness of water of the order of a metre or more in depth rather than the usual sub-glacial film of a fraction of a millimetre. Thus the reflexion coefficient suggests that we are dealing with a substantial layer of water, rather than an exceptionally smooth

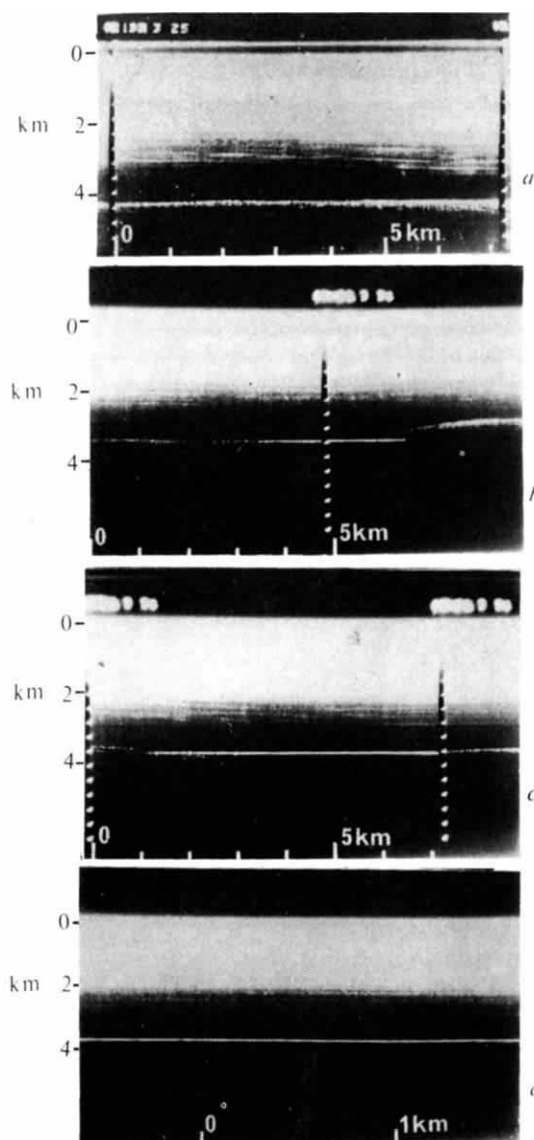


Fig. 2 *a*, Typical strong echo from bedrock, showing normal fading, and some downward extension of the trace due to non-vertical echoes. The column of white dots gives a time reference at 5 μ s intervals; *b*, clearly defined example of a smooth reflecting surface under the ice, showing a steep scarp at one edge and a more gradual slope at the other; *c*, further example, with more symmetrical sloping rock margins than shown in Fig. 2*b*; *d*, section from Fig. 2*c* with the horizontal scale expanded four times, illustrating the slow rate and small amplitude of fading.

Table 1 Location and Width of Sub-Ice Lakes

Site No.	Latitude S	Longitude E	Depth of ice cover (m)	Width along flight line (km)
1	76° 42'	122° 45'	3,302	4
2	73° 15'	157° 12'	2,830	5
3	77° 28'	123° 42'	3,600	2
4	74° 08'	124° 35'	4,094	7
5	72° 41'	127° 21'	3,889	4
6	72° 18'	123° 54'	3,254	3
7	77° 59'	122° 43'	3,666	3
8	72° 55'	156° 23'	2,840	2
9	76° 04'	128° 14'	2,969	2
10	75° 56'	127° 21'	3,231	15
11	75° 43'	126° 05'	3,149	4
12	75° 00'	121° 52'	3,057	1
13	75° 54'	122° 06'	3,517	4
14	75° 10'	126° 30'	3,436	3
15	73° 55'	120° 21'	4,047	5
16	76° 42'	136° 23'	3,101	2
17	76° 20'	87° 45'	4,200	7

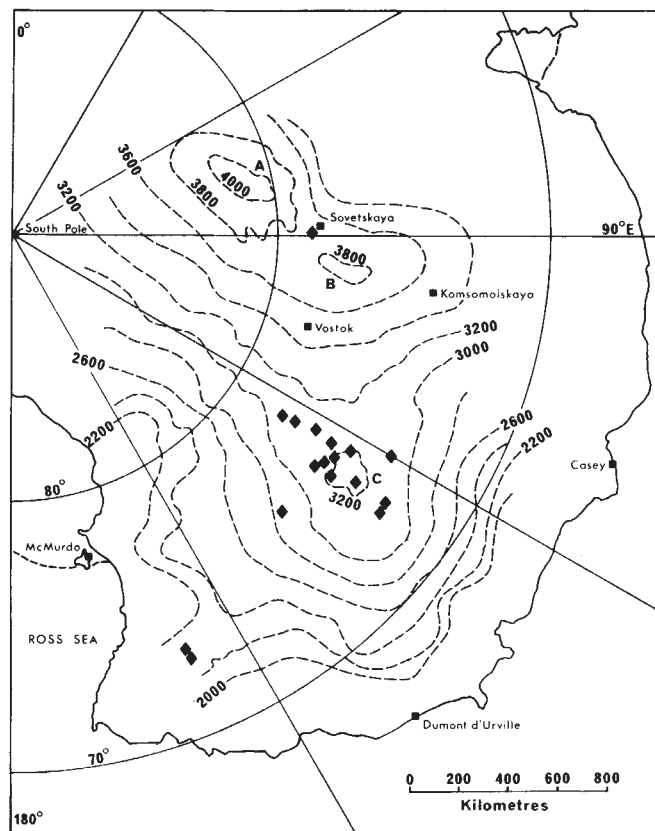


Fig. 3 A map of the surface contours of East Antarctica. The lake sites (as in Fig. 1) are seen to occur in regions of low surface slope.

rock floor beneath the ice. Furthermore, the immediate surroundings of the smooth, near-horizontal sub-glacial echoes, where visible on the records as in Fig. 2b and c, frequently suggest basins with the surrounding rock sloping down to the level of the smooth reflectors, which we will now term lakes.

The location of the sub-ice lakes is shown by the diamond shapes on the map in Figs 1 and 3, the latter showing their distribution in relation to surface contours of the ice sheet. The surface contours have been determined as part of our sounding programme under the International Antarctic Glaciological Project⁵. The results, which have not yet been published in detail, show that in addition to the highest central dome of the ice sheet (A in Fig. 3), two secondary domes, B and C, exist in the area we have studied. Such domes will also be centres of outflow of the ice, so that in their vicinity surface slopes are low and the velocity of ice movement is small.

Sixteen lakes have been discovered in the 1971–72 records in addition to one from the 1967–68 season near Sovetskaya station, shown in Fig. 1 of ref. 6, for which a tentative explanation in terms of a sub-ice lake was made. The geographical locations of all echoes are shown in Fig. 3. Most are grouped round the centre of outflow of ice at 75° S, 123° E (dome C), while the two near longitude 157° E and the one near Sovetskaya station are near saddles in the ice surface. Further details are given in Table 1.

The existence of meltwater beneath this part of the ice sheet is not in agreement with the computer calculations of Budd, Jenssen and Radok¹⁰ who estimate basal temperatures of –20° C to –30° C over a region covering the main group of specular reflexions. Such calculations are based on earlier estimates of ice thickness, geothermal heat flux and surface accumulation rates. The latest information on these parameters given by the present radio-echo programme and the surface traverse of French scientists, both forming part of the International Antarctic Glaciological Project, indicates that basal melting can occur around dome C when ice depths are about 2,900 m or more (D. Jenssen, unpublished). Similar

conclusions seem reasonable for sub-ice lakes at the other two localities. In making these calculations, the major uncertainties arise in estimating the geothermal heat flux, and in assuming that the true temperature distribution can be approximated by a steady-state system. It is satisfactory that calculations based on the newest data appear compatible with our interpretation of the radio-echo observations.

Although the presence of basal water seems reasonable, we have yet to explain why it should accumulate in certain localities since the pressure gradient beneath a glacier normally serves to drive out sub-glacial water. In addition to the other factors discussed in detail by Weertman⁸ the flow of water is controlled mainly by two factors: the ice thickness gradient, and the basal slope. Looking at the problem in two dimensions, where x is a distance measured along the line of flow, the direction of flow, F , can be derived from the balance of pressure gradients:

$$F \propto (-\rho_w g \beta_x - \rho_i g \partial H / \partial x)$$

where ρ_i and ρ_w are the respective densities of ice and water, H is the ice depth, β_x is the basal slope and g the acceleration due to gravity. If α_x is the surface slope

$$\partial H / \partial x = \alpha_x - \beta_x$$

Taking ρ_i and ρ_w such that $(\rho_w - \rho_i) / \rho_i = 1/10$

$$F \propto (-\beta_x - 10\alpha_x)$$

Thus flow in the positive sense due to a negative surface slope α_x can be prevented by a basal slope in the opposite sense and greater by a factor of at least 10, thus forming a lake. Such conditions are most likely to be found in localities where surface slopes are small, such as domes and saddles on the ice sheet. This explains the distribution of sub-ice lakes shown in Fig. 3, taken in conjunction with the above discussion of basal melting. We do not expect to find lakes beneath domes A and B of Fig. 3, since basal temperatures here are calculated as well below freezing point.

Once sub-ice basins have filled to overflowing, there may well be some interconnection between lakes by a sub-ice film of water, or by sub-ice channels, though according to Weertman⁸, the latter is less likely. It is, however, likely that much of the sub-ice water will be refrozen to the base of the ice further out from the centre of the ice sheet. It has been estimated⁷ that this effect takes place in Byrd Land and seems likely in Greater Antarctica on the basis of temperature calculations^{9,10}. Nevertheless, the possibility remains that the deeper parts of the sub-glacial floor may permit some drainage from sub-ice lakes to the sea.

The presence of lakes beneath an ice sheet at least several million years old presents a different problem to that of the bodies of water that appear to be trapped near the Ross Ice Shelf–Byrd Land boundary described in Robin, Swinbank and Smith⁶. Whereas the latter may be of a relatively recent origin in geological or glaciological terms, the latest evidence from Hayes *et al.*¹¹, obtained during deep sea drilling in the Ross Sea by the Glomar Challenger, suggests that the main Antarctic ice sheet changed little in size since a retreat some 5 m.y. ago. It may be wrong to suppose that basal temperature conditions have remained the same over this period, in which case the sub-ice lakes may be much younger than the ice sheet. But we should consider if the sub-ice lakes could be as old as 5 m.y. or more. One must take into account the melting of ice that will occur at the base of the ice if the lakes are to persist over such long periods. The available geothermal and frictional heat will limit such melting to less than a centimetre per year and it is likely to be of the order of 1 mm yr⁻¹. Even such melting over a period of several million years would melt an ice column totalling several thousand metres in thickness. As this will be basal ice which normally carries morainic material, we should expect such material to be deposited in the sub-ice lakes and, in the course of time, to fill them. For the lakes to persist, we must postulate a low rate of deposition of morainic material from the melting ice. Two factors may

assist here. The first is the possibility of a low rate of erosion near the centre of the ice sheet and the second is that the rate of transport of the lowest layers of ice across the lakes is very low. Such effects are to be expected near a centre of outflow and may be another reason for the concentration of sub-ice lakes in such areas.

Another question which is raised by the presence of such lakes is that of biological conditions in the water. The survival of any life forms existing in these regions before the glaciation seems unlikely, considering the environmental changes which must have taken place. But once basal melting of the ice commenced, it would provide a slow, steady influx of microorganisms deposited in the past on the surface of the ice. The geothermal heat flux provides a source of energy, and, with the oxygen dissolved in the ice, it is conceivable that the water provides a refuge for any such microorganisms which have survived the downward passage through the ice, which will have taken 10^5 yr or more.

The practical need for knowledge of conditions at the base of the Antarctic ice sheet has been given special emphasis by the recent proposal of Zeller, Saunders and Angino¹² for dumping radioactive wastes on the ice sheet.

It has been indicated that the possibility of a connexion between the sub-ice water and the sea cannot be ruled out, and this must call into question any proposal which assumes the base of the ice to be totally isolated from the habitable world. Further, the extent of basal melting is critically dependent on the heat influx at the base of the ice. The drastic local disturbances caused by the heat output from such dumps, and their effect on the dynamics of the ice sheet, must be better understood before such proposals can be properly assessed.

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G. K. A. OSWALD
G. DE Q. ROBIN

Scott Polar Research Institute,
Cambridge

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- ¹ Evans, S., and Smith, B. M. E., *J. scient. Instrum.*, Series 2, **2**, 131 (1969).
- ² Robin, G. de Q., Evans, S., and Bailey, J. T., *Phil. Trans. R. Soc.*, **A265**, 437 (1969).
- ³ *Antarctic Geology and Geophysics* (edit. by Adie, R. J.) (Universitetsforlaget, Oslo, 1972).
- ⁴ Harrison, C. H., thesis, Univ. Cambridge (1972).
- ⁵ International Antarctic Glaciological Project, *Polar Record*, **15**, 829 (1971).
- ⁶ Robin, G. de Q., Swinbank, C. W. M., and Smith, B. M. E., *IASH Publ.*, **86**, 97 (Hanover, New Hampshire, 1970).
- ⁷ Budd, W. F., and Radok, U., *Rep. Prog. Phys.*, **34**, 1 (1971).
- ⁸ Weertman, J., *Rev. Geophys. Space Phys.*, **10**, 287 (1972).
- ⁹ Zotikov, I., *Mat. Glyats. Issled. Khron. Obs.*, **10**, 150 (1964).
- ¹⁰ Budd, W. F., Jessen, D., and Radok, U., *Meteorology Dept.*, Publ. No. 18 (Univ. Melbourne, 1971).
- ¹¹ Hayes, D. E., *et al.*, *Geotimes*, **18** (6), 19 (1973).
- ¹² Zeller, E. J., Saunders, D. F., and Angino, E. E., *Bull. atom. Sci.*, **29**, (1), 4 (1973).

Recent Faulting along the Mediterranean Coast of Israel

Two hypotheses explaining the origin of the present coastline of Israel, one postulating a tectonic, the other merely a wave-abrasion mechanism, have long been the subject of controversy. The arguments supporting a tectonic origin of the coastline are its linearity and the fact that it consists of

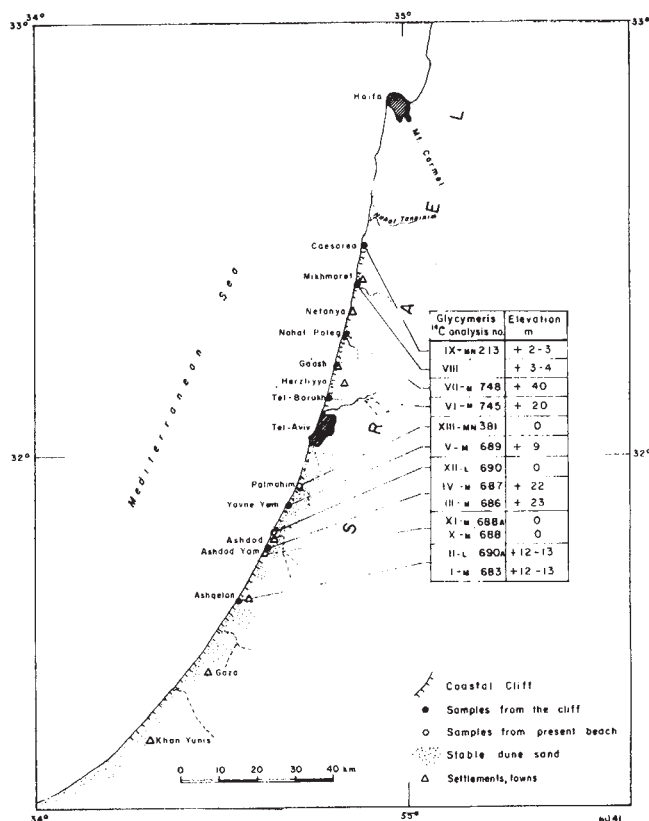


Fig. 1 Location map.

an almost continuous line of low cliffs from Khan Yunis in the south to Mount Carmel in the north. The chief argument for an abrasional origin was the dominant trend of the waves coming from the west, perpendicular to that part of the coastline.

Systematic observations made by us since 1961 along the coastline, the adjacent cliffs and the offshore littoral zone from Gaza to Caesarea have revealed the following:

(A) At various localities (Fig. 1), horizons of naturally deposited marine shells are found at different elevations (3 to 40 m), on or near the top of the cliffs. In some places these beds are found as far as several hundred metres landward from the shoreline (Ashdod, Gaza). The beds are dominated by the aragonite-secreting pelecypod *Glycymeris violacescens* (Lmk.) and are found either on the surface or interbedded in the sandy sediment, in places forming a layer up to 1 m thick. Usually the shells overlie either kurkar (cemented aeolianite) or hamra (red loam) of upper Pleistocene to Holocene age. At several localities (such as Ashqelon, Mikhmoret and Caesarea) the beds are found overlying ancient sites of Roman to Crusader times. In some cases, pottery sherds of various ages (the latest of which is Mediaeval-Crusader) are interbedded and imbricated within the *Glycymeris* accumulations. The fact that many of the sherds have been rounded through wave action into pebble-like fragments is of special importance.

(B) Sand dunes which occur along most of the coast of Israel terminate abruptly north of Nahal Tanninim (Fig. 1). Most of these sand dunes are today inactive, partly stabilized by vegetation. In the south (Ashdod, Ashqelon, Gaza) these dunes cover settlements as young as Early Moslem, which were originally built on red loam (hamra) plains. In the central and northern parts of the discussed area, the coastal cliffs are much higher (up to 50 m above sea level) than in the south, and the sand dunes are found on top of the cliffs (Herzliya, Netanya); at Gaash, for example, they cover a Byzantine settlement located 41 m above sea level. These relatively high, near vertical cliffs are only 10 to 20 m from

the shoreline and there is not sufficient space for the prevailing westerly winds to drive the sand upward on the cliffs. These sands are known to be derived originally from the Nile River and transported by sea currents.

(C) The seafloor from the coastline to several hundred metres seawards is rocky in patches. From Tel-Aviv to Mount Carmel these patches of rocks may reach sea level, and their westward termination is well marked on air photographs as a long straight line.

The submerged breakwater of the Herodian harbour of Caesarea¹, built about 10 BC, is crossed by this straight line (Fig. 2). Lead joints cast by the masons at that time in order to strengthen the walls were found *in situ* during submarine archaeological studies² at the entrance of the breakwater, west of the rocky bottom, more than 10 m below sea level (Fig. 2). As the lead joints must have been cast above sea level, this part of the harbour must have subsided by at least 10 to 15 m since its construction.

(D) The absolute ages of the specimens of *Glycymeris violacescens* from several localities (Table 1) were determined by the ¹⁴C method. The shells were first checked by X rays to ensure that they remained aragonite and that no recrystallization had occurred. The age homogeneity of the specimens in every sample was verified by its ¹⁸O/¹⁶O ratio³. Table 1 gives the results of the ¹⁴C analyses (made at the Weizmann Institute) as well as sample descriptions, localities and elevations. The absolute ages of all the samples from the different elevations above sea level range from 2,100 to 3,800 yr BP. But some of them were found interbedded and imbricated with pottery sherds as young as 700 yr BP, and accordingly that dates the deposition and accumulation of these marine shells.

This apparent age discrepancy prompted several ¹⁴C age analyses of *G. violacescens* samples from the huge accumulations of loose specimens which occur near the present waterline, essentially at sea level. These shells, which were formerly assumed to be recent, yield ages ranging from 1,150 to 1,500 yr BP (Table 1, samples X to XIII). This age span might represent a delay time during which the shells were transported from the depth at which they usually live⁴ to the present beach. There seems no doubt that all the

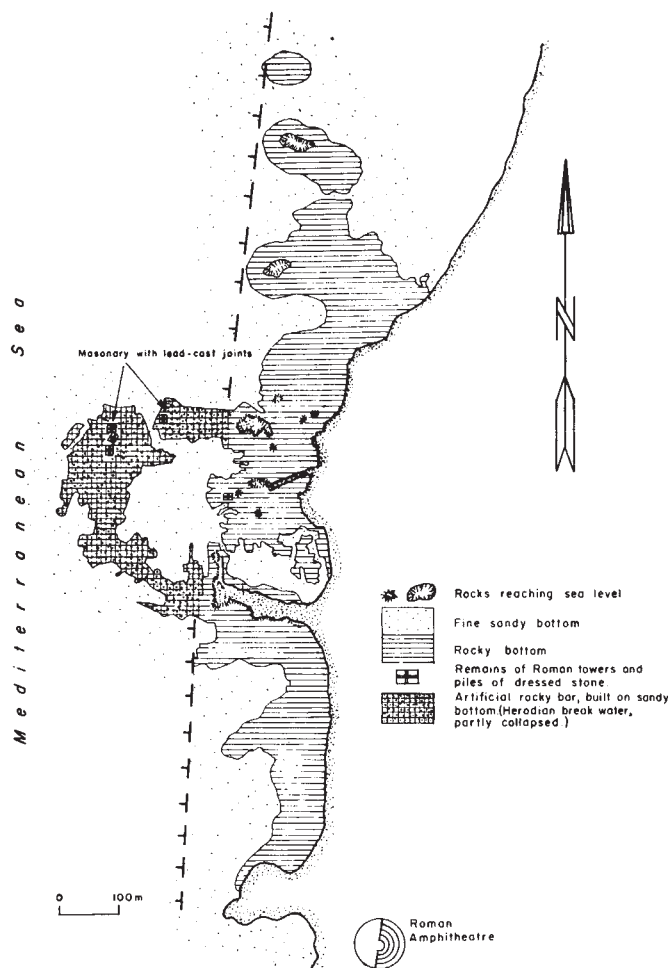


Fig. 2 Bottom physiography of the Herodian harbour in Caesarea (after Linder and Leenhardt¹) as modified by the authors—based on air photographs and seismic profiling results, showing the termination of the rocky bottom along the presumed fault line.

Table 1 ¹⁴C Ages, Localities and Description of *Glycymeris* Shells Analysed

Sample No.	Field No.	Location	Coordinate	Elevation (m)	Age BP *	Description
Samples from the coastal cliff						
I	M683	Ashqelon	1074/1199	12–13	2,610 ± 130	Bedded shells, mediaeval pottery sherds, gravel on calcareous aeolianite ridges
II	L690A †	Ashqelon	1074/1199	12–13	2,500	Bedded shells, mediaeval pottery sherds, gravel on calcareous aeolianite ridges
III	686	Ashdod-Yam	1140/1314	23	2,120 ± 200	Shells on surface of red sandy loam
IV	687	Ashdod-Yam	1140/1314	22	2,360 ± 260	Shells within sandy loam
V	689	Yavne-Yam	1180/1400	9	3,800 ± 210	Bedded shells within sand dune
VI	745	Tel-Barukh	1305/1710	20	2,920 ± 220	Shells on surface of red sandy loam
VII	748	Nahal Poleg	1355/1906	40	3,800 ± 150	Shells on surface of red sandy loam
VIII		Mikhmoret	1356/2007	3–5	2,800 ± 360	Bedded shells with Byzantine pottery sherds and gravel deposited on calcareous aeolianites
IX	231	Caesarea	1397/2098	3–5	3,300 ± 160	Shells on surface with mediaeval pottery sherds and gravel
Samples from the present beach						
X	M688	Ashdod-Yam	1138/1315	0	1,500 ± 300	Shells from accumulation on present beach, least corroded
XI	M688A	Ashdod-Yam	1138/1315	0	1,520 ± 200	Shells from same accumulation on present beach, most corroded
XII	L690B †	Ashdod	1067/1190	0	1,300	Shells from accumulation on present beach
XIII	MN381	Palmahim	1221/1491	0	1,150 ± 120	Shells from accumulation on present beach
Living specimens						
XIV	RT373	Palmahim		0	0	<i>Monodonta</i> sp.
XV	394	Principality of Monaco	–20–30	–20–30	0	<i>Glycymeris violacescens</i>

* These results represent the time since the death of the molluscs. But the time of deposition of the shell beds might be regarded as appreciably younger, as may be deduced from results obtained on present beach samples (X–XIII) and the ages of interbedded pottery sherds (see text).

† Collected by D. Neev in 1961. Preliminary results by Lamont Radiocarbon Laboratory, Palisades, NY, 1962.

fossils mentioned incorporated a zero ^{14}C age initially. This is shown by the normal (zero) ^{14}C age found in the present day samples of the live material (Table 1, samples XIV and XV); sample XIV is a gastropod (a *Monodonta* sp.), collected alive from the Israel coast, and sample XV comprises shells of *G. violaceus*, collected at Monaco, where it flourishes at a depth of about 30 m.

Though great quantities of *G. violaceus* shells are very common on the Israeli beaches, only a few live adult specimens were ever found, in spite of persistent searching and dredging. Their present day absence may be due to ecological reasons which influence their distribution in this part of the Mediterranean.

Avnimelech⁵, who discussed the phenomenon of the *Glycymeris* beds, related them to the Flandrian transgression (6,000–12,000 yr ago). Our data suggest that the beds were deposited much more recently and that they have been lifted to their present position due to a tectonic mechanism, and not a eustatic one. Moreover, to assume even a 10 m rise of the sea level means that the seawater would have reached far inland, covering large parts of the lowland and valleys. No evidence of such an extensive transgression has been found. The effects of the tide in this region (some 30–40 cm) can be neglected.

It seems that the entire coastal zone, at least from Ashqelon to Caesarea, was downwarped and submerged under the Mediterranean waters. This submerged position was maintained for some time during which the marine shells, rounded pebbles and coastal sand dunes accumulated. Later, the offshore littoral zone west of the fault line remained submerged, whereas the area east of the fault (along the present coastline) was uplifted to its present position. This tectonic event probably occurred later than 700 yr BP (mediaeval age of the latest interbedded pottery sherds, the submergence of the Herodian breakwater and the dunes and molluscan shells covering the early Moslem sites). But the earliest age of this event could not be more than 3,800 yr BP, the age of the oldest marine shells.

We assume that these complicated tectonic movements are part of a more regional process, due to which the coastal plain, shelf and slope of Israel were arched and the coastal zone was submerged. The tension created as a result of this arching was probably released by the uplifting of the eastern side of the fault, along the present coastline.

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D. NEEV
N. BAKLER
S. MOSHKOVITZ

*The Geological Survey of Israel,
Jerusalem*

A. KAUFMAN
M. MAGARITZ

*Department of Isotope Research,
The Weizmann Institute of Science, Rehovot*

R. GOFNA

*Israel Department of Antiquities,
Jerusalem*

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¹ Linder, E., and Leenhardt, C., *Revue Archaeologique*, 47 (1964).

² Fritsch, C., and Ben-Dor, I., *The Biblical Archaeologist*, 24, 50 (1960).

³ Magaritz, M., and Kaufman, A., *Nature*, 243, 462 (1973).

⁴ Riedl, R., *Fauna und Flora der Adria* (Verlag Paul Parey, 1970).

⁵ Avnimelech, M., *Quaternaria*, 6, 479 (1962).

Methylmercury in Estuarine Sediments

INDUSTRIAL mercury discharges into natural waters have been effectively reduced during the past several years in the United States. In areas such as Mobile Bay, which previously received sufficient quantities of anthropogenic mercury effluents to require a ban on commercial fisheries, it is now critical to determine if the residual contaminated sediments are a reservoir of mercury compounds which could be detrimental to the fishery on a long term basis. It is well established that mercury accumulates in particulate material^{1,2} and may subsequently be transferred from sediment to fish³. Laboratory studies⁴ suggest that diagenetic processes in natural sediments can transform inorganic mercury to mono and dimethylmercury, increasing the pollution hazard. The methylated forms of mercury have been found to be more toxic to organisms than inorganic or phenyl mercury compounds^{5,6}. Here we report the first published results on the concentration and distribution of methylmercury compounds in natural sediments from polluted and unpolluted coastal environments.

Our study was designed to include a wide range of typical estuarine environments discharging into the Gulf of Mexico. Surface sediments were collected and analysed from the Mississippi Delta, Mobile Bay and the Florida Everglades. Several short gravity cores collected in Mobile Bay were also analysed to study vertical dispersion of mercury in the sediment. All the sediment samples were frozen immediately after collection and returned to the laboratory for the determination of total mercury, methylated mercury and total organic content. Total mercury was determined by flameless atomic fluorescence⁷. The method of Rudling⁸ was slightly modified to determine the combined monomethyl and dimethylmercury in sediments. In our procedure the methylmercury was converted to its bromide complex rather than to its chloride complex. A Varian 2100 gas chromatograph with a Ni-65 electron capture detector was used for the analyses. Standard addition experiments were performed to ascertain the efficiency of extraction (approximately 80%). The precision measured on four replicate samples was $\pm 18\%$. The limit of detection was approximately 0.02 ng g^{-1} of material. Sample organic content was taken as the difference in weight after combustion at 600°C for 4 h.

Table 1 Methylmercury, Total Mercury and Organic Matter in Surface Sediments

Location	Station	Total Hg (ng g^{-1})	MeHg (ng g^{-1})	% MeHg of total Hg	% organic carbon
Mobile Bay	16	210	0.06	0.03	5.5
	19	600	0.19	0.03	11.4
Mississippi River	1	140	<0.02	<0.01	0.5
	5	80	<0.02	<0.01	2.4
	10	510	0.05	0.01	6.9
	15	570	0.05	0.01	9.4
Everglades	4	120	0.06	0.05	2.0
	14	120	0.08	0.07	11.1
	17	490	0.12	0.03	69.0

Surface sample data from the three areas are presented in Table 1. The methylmercury concentration varied from less than 0.02 ng g^{-1} to 0.19 ng g^{-1} , the high value being in the estuarine sediments from Mobile Bay. The most interesting aspect of the data is the fact that the methylmercury content never represents more than 0.07% of the total mercury present; the average is 0.03%. The four samples from the Mississippi River have exceedingly low methylmercury concentrations whereas samples from the Everglades nad Mobile Bay are comparable. This is of interest since Mobile Bay received chloralkali effluents until recently whereas there

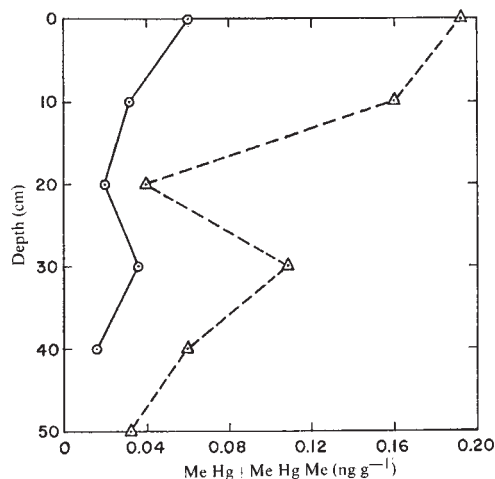


Fig. 1 Distribution of methylmercury with depth in sediment cores from Mobile Bay. $\circ$, Station 16; Δ , station 19.

are no known anthropogenic mercury discharge sources in the vicinity of the Everglades.

Measurements of methylmercury with depth in two cores from Mobile Bay were also made. Both cores were reducing except for the upper 4 to 5 cm. Figure 1 shows that the methylmercury values decrease with depth to minima of approximately 0.02 ng g^{-1} . Figure 2 shows that the methylmercury concentrations vary from 0.04% to 0.006% of the total mercury. Jernelov⁹ suggests that the rate of methylation is considerably reduced in anoxic systems, which may partially explain the low methylmercury concentrations observed in the sediments studied.

Other studies in our laboratory have demonstrated an enrichment of up to thirty times of dissolved mercury in sediment pore water relative to overlying estuarine water¹⁰. Our results indicate that methylation processes do not play a major role in the postdepositional migration of mercury in estuarine sediments. Thermodynamic calculations suggest that polysulphide complexes with mercury may be the basis of a mechanism producing the enrichment of mercury in sediment pore water¹⁰. Release of mercury into pore water combined with diffusion or sediment mixing by dredging and wave action provides a mechanism for the detoxification of contaminated sediments. Future studies should concentrate on the kinetics of mercury-sulphide systems in simulated pore water environments.

The presence of methylated mercury as the predominant form of mercury in many aquatic animals^{11,12} has resulted in an emphasis in many bioassay studies on direct exposure of

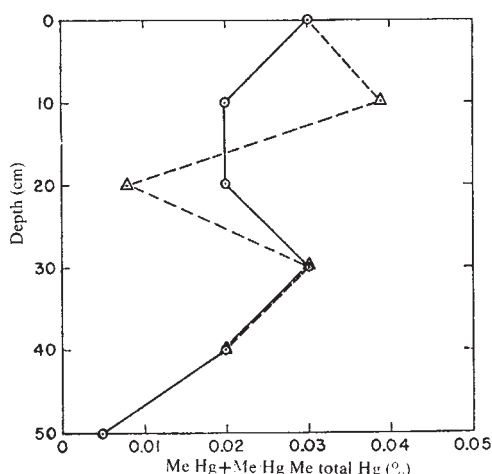


Fig. 2 Distribution of methylmercury as a percentage of total mercury in sediment cores from Mobile Bay. $\circ$, Station 16; Δ , Station 19.

test organisms to methylmercury in water. Our results suggest that future toxicological studies should emphasize biochemical mechanisms for synthesis of methylmercury from inorganic mercury species.

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ANDERS W. ANDREN
ROBERT C. HARRISS

Oceanography Department and the
Marine Laboratory,
Florida State University,
Tallahassee, Florida 32306

Received June 11, 1973.

- ¹ Klein, D. H., and Goldberg, E. D., *Environ. Sci. Tech.*, **4**, 765 (1970).
- ² *Geol. Surv. Prof. Pap.*, 713 (US Government Printing Office, Washington DC, 1970).
- ³ Gillespie, D. C., *J. Fish. Res. Bd Can.*, **29**, 1035 (1972).
- ⁴ Jensen, S., and Jernelov, A., *Nature*, **223**, 753 (1969).
- ⁵ Harriss, R. C., White, D. B., and Macfarlane, R. B., *Science*, **N.Y.**, **170**, 736 (1970).
- ⁶ Lofroth, G., *Swedish natn. Sci. Res. Council Ecol. Res. Comm.*, **Bull.**, No. 4 (1969).
- ⁷ Muscat, V. I., Vickers, T. J., and Andren, A. W., *Analyt. Chem.*, **44**, 218 (1972).
- ⁸ Rudling, L., Mimeographed procedure from Swedish Air and Water Pollution Research Laboratory, Stockholm (1970).
- ⁹ Jernelov, *The Changing Chemistry of the Ocean, Nobel Symposium 20* (edit. by Dyrssen, D., and Jagner, D.) (1972).
- ¹⁰ Lindberg, S., thesis, (Florida State Univ., 1973).
- ¹¹ Johansson, B., Ryhage, R., and Westoo, G., *Acta Chem. Scand.*, **24**, 2349 (1970).
- ¹² Zitko, V., Finlayson, B. J., Wildish, D., Anderson, J., and Kohler, A., *J. Fish. Res. Bd Can.*, **28**, 1285 (1971).

Interstellar Radio Communication and the Frequency Selection Problem

THE largest microwave radio telescope on Earth, at the Arecibo Observatory of the National Astronomy and Ionosphere Center, will soon have the capability of communicating with an identical radio telescope, if such exists, anywhere in the Galaxy. But such communication assumes some previous agreement between the transmitting and receiving civilizations, or mutual discovery of the chosen radio frequency, bandpass, information rate and each other's location.

The question of frequency choice is a prime one, for once the frequency and location are identified, the bandpass and information rate can readily be determined empirically. This is a difficult problem. For example, with even the exceptionally wide bandpass of 1 MHz, there are some 10^5 possible channels to be examined over the entire radiowave spectrum. This problem could be greatly eased if one could establish one or a few frequency channels which are "natural"—that is, which are specified clearly by an astronomy, chemistry or physics which is shared in common between transmitting and receiving civilizations.

The first suggestion of such a natural frequency¹ was the 1,420.405 MHz hyperfine transition of neutral atomic hydrogen, the most abundant atom in the Universe. The few serious searches for interstellar radio communication which have been performed have been restricted to this frequency²⁻⁵. To date these few searches have yielded negative results. But even under rather optimistic assumptions, the mean distance to the nearest technical civilization works out to be several hundred light years, and the corresponding number of stars which must be searched is $\sim 10^6$ (ref. 6). Thus negative results in the examination of $\sim 10^5$

stars do not constitute a significant finding, even under optimistic assumptions. The anticipated probability of success in the efforts to date is about 10^{-4} . This underlines the magnitude of effort called for, and the great possible advantages of using "natural" frequencies.

It is useful to consider whether alternative and more attractive natural frequencies exist. In the galactic plane, the 1,420 MHz line is noisy, which decreases its attractiveness. Under not unreasonable assumptions, it may be the very long-lived and more distant civilizations which dominate interstellar radio communications traffic⁷⁻⁹. Because of the problem of distance, a clearer frequency may be the communications channel of choice.

There are now many other interstellar microwave lines known, in which the confusion due to emission or absorption in the line is less in some cases than for the hydrogen hyperfine transition; but the large number of such lines makes a preferred choice difficult. The first or second overtone of the 1,420 MHz line is one answer to this problem⁶.

Here we propose two alternative natural frequencies for interstellar radio communication based on two quite different and, we feel, attractive arguments. Noise in sophisticated interstellar radio links will have four components: the galactic noise; the 2.7 K isotropic blackbody background radiation; the quantum noise of the radiation itself; and perhaps noise from a planetary atmosphere. Present information^{10,11} places the minimum sky noise temperature from all four of these sources at about 5 K centred at a frequency of a few GHz. This is not far from both the hydrogen hyperfine transition and the principal hydroxyl microwave transition frequency of 1,667.358 MHz, and thus close to the frequencies associated with the components of the water molecule.

Life on Earth is in many ways water based. Even when facing a caveat to avoid water chauvinism, there are powerful biochemical and cosmic abundance arguments that extraterrestrial life will, in the main, also use water as a solvent system¹²⁻¹⁵. Accordingly, the proximity of the principal microwave transitions of H and OH to the minimum sky noise region has suggested to Oliver^{10,11} that the preferred communications channel is this "water hole": between 1,420 and 1,667 MHz. Even here, however, there still remains a multitude of possible channels. We therefore point out that there is a well defined "natural" frequency in the water hole: a frequency selected by the centre of mass of the water molecule itself.

This frequency can be obtained by assigning 1,420.405 MHz to the H atom and 1,667.358 MHz to the OH radical. The natural frequency is then the one associated with the centre of mass of the molecule, and is given by increasing the OH frequency or wavelength by an amount M_H/M_{OH} times the frequency or wavelength difference, respectively, between the H and OH transitions.

There is, of course, an ambiguity caused by the fact that one may relate the frequencies or the wavelengths to the spatial geometry of the molecule. Either approach, however, gives nearly the same answer, the frequency analogy leading to a frequency associated with the centre of mass of 1,653.541 MHz and the wavelength analogy leading to a frequency for the centre of mass of 1,651.295 MHz. It is probably more reasonable to choose the second, wavelength-derived, frequency, since one is there using lengths as analogues to lengths, but this rationale is primarily aesthetic and possibly provincial. Probably the most objective choice, since it avoids making a selection between the frequency and wavelength analogue, is to take the mean, either arithmetic or geometric, between the two frequencies, which gives the "natural" frequency of 1,652.418 MHz.

If there are other solvent systems for some subset of extraterrestrial organisms, it may be that they communicate on a correspondingly weighted channel for the transition

frequency for their principal solvent system, ammonia for example. If there are any organisms with solvent systems based on simple hydrocarbons¹⁵, they would be unable to use this scheme since there are no permitted microwave transitions for such molecules.

There is also an entirely different "natural" frequency uniquely specified by the intersection of two fundamental sources of noise: the blackbody background and the quantum noise of radiation. The corresponding frequency kT_{bb}/h , where k and h are the Boltzmann and Planck constants respectively, is 56 GHz for $T_{bb}=2.7$ K. The frequency cannot be specified to higher precision because of present uncertainties in the precise values of T_{bb} . This channel, at a wavelength of about 5.4 mm, is unavailable from the surface of the Earth because of the strong molecular oxygen lines nearby. Communication on this channel requires radio telescopes in space which will probably be available soon (especially on a stellar evolutionary time scale). The galactic sky background at 56 GHz is only slightly noisier than in the 1.65 GHz channel.

The 56 GHz channel has the provocative property of being determined simultaneously by quantum mechanics and cosmology. The same frequency for this "quantum/cosmology" channel should be derived for any civilization within the Galaxy. A transmitting civilization of Type III (ref. 16) in a distant galaxy will, however, have transmitted its signal to the Earth at an earlier cosmological epoch, when T_{bb} was larger than is measured today. This will tend to increase the "natural" frequency. The cosmological Doppler effect will, however, tend to decrease the frequency. Were such a signal received in the millimetre waveband but displaced from 56 GHz, its frequency might even be used to deduce the distance and epoch of transmission of the signal.

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F. D. DRAKE
CARL SAGAN

*National Astronomy and Ionosphere Center,
Cornell University,
Ithaca, New York*

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¹ Cocconi, G., and Morrison, P., *Nature*, **184**, 844 (1959).

² Drake, F. D., *McGraw-Hill Yearbook of Science and Technology*, 384 (1961).

³ Troitsky, V. S., in *Communication with Extraterrestrial Intelligence* (edit. by Sagan, C.) (MIT Press, Cambridge, 1973).

⁴ Verschuur, G., *Icarus*, **19**, 329 (1973).

⁵ Zuckerman, B., and Palmer, P., Paper given at URSI meeting, Socorro, New Mexico, January 1973.

⁶ Shklovskii, I. S., and Sagan, C., *Intelligent Life in the Universe* (Holden-Day, San Francisco, 1966).

⁷ Kardashev, N. S., in *Extraterrestrial Civilizations: Problems of Interstellar Communication* (edit. by Kaplan, S. A.) (Nauka, Moscow, 1969).

⁸ Drake, F. D., in *Communication with Extraterrestrial Intelligence* (edit. by Sagan, C.) (MIT Press, Cambridge, 1973).

⁹ Sagan, C., *Icarus*, **19**, 350 (1973).

¹⁰ Oliver, B., *Project Cyclops, A Design Study of a System for Detecting Extra-Terrestrial Intelligent Life* (NASA Document CR11445, 1971).

¹¹ Oliver, B., in *Communication with Extraterrestrial Intelligence* (edit. by Sagan, C.) (MIT Press, Cambridge, 1973).

¹² Henderson, L. J., *The Fitness of the Environment* (Macmillan, New York, 1913).

¹³ Blum, H. F., *Time's Arrow and Evolution* (Princeton University Press, Princeton, 1931).

¹⁴ Sagan, C., *Life* (*Encyclopedia Britannica*) (1970).

¹⁵ Pimentel, G. C., et al., in *Biology and the Exploration of Mars*, Publication 1296 (National Academy of Sciences/National Research Council, 1966).

¹⁶ Kardashev, N. S., *Transmission of Information by Extraterrestrial Civilizations*, *Soviet Astr.*, **8**, 217 (1964).

BIOLOGICAL SCIENCES

Cell Cycle Regulation of Mating Type Alleles in the Smut Fungus *Ustilago violacea*

THE sexual cycle of the smut fungus, *Ustilago violacea*, is initiated by conjugation between uninucleate, yeast-like haploid sporidia of opposite mating type. The mating type is determined by a pair of alleles (a_1 and a_2). Mating is achieved by the co-operative assembly of a tube between the paired sporidia. After copulation the haploid nuclei remain separate and a dikaryotic mycelium is established. This dikaryon is an obligate parasite of a host plant (family Caryophyllaceae). Nuclear fusion (karyogamy) is induced in hyphae inhabiting the anthers of a host plant and a diploid brandspore is formed. When the brandspore germinates its nucleus divides meiotically to re-establish the haploid sporidial phase¹. In this basidiomycete plasmogamy and karyogamy are temporally separated and the mechanisms governing each can be analysed separately. We wish to report here observations on the relationship between the cell cycle and cellular morphogenesis (manufacture and assembly of the conjugation organelle) in the sporidial phase of the life cycle. We have evidence that mating depends on the stage in the cell cycle of conjugating sporidia. The mating type alleles are differently regulated during the cell cycle, as cells bearing allele a_1 seem to be under stringent control and copulate only during the G_1 phase of a cell cycle, while cells bearing the dominant a_2 allele have a relaxed control and can initiate conjugation during most of the cell cycle.

In *U. violacea*, conjugation is initiated when cells of opposite mating type are brought into contact on a medium free of nutrients. Mating consists of a synchronous series of biochemical and morphological events. During the first 2–4 h of mating distinct morphological changes are not observed, but experiments in progress indicate that during this period the genes governing mating are transcribed and translated. Between 4 and 8 h after the initiation of conjugation the mating organelle is assembled and later elongates to the mature mating configuration¹.

We studied the influence of the cell cycle on the mating activity of a mixed culture of the two mating types using sucrose gradients to synchronize the sporidia². We inoculated the top fraction from a sucrose gradient that contained less than 10% budded cells (the logarithmic population has 50–60% budded cells) into complete medium and followed DNA replication in that culture by measuring ¹⁴C-adenine incorporation as the culture grew out synchronously. We removed cells from the synchronous population at fixed intervals and tested them for conjugation by washing them and transferring them to the surface of a non-nutrient agar plate. Random cultures show 40–50% conjugation 12–24 h after initiation of mating while the early G_1 fractions from synchronous cultures always exceed this value and generally reach 60–70% conjugation. As the synchronous population passes through G_1 , conjugation decreases steadily to a low of about 20% during S phase and G_2 , then rises to a second peak after the culture passes through mitosis and enters G_1 . A typical result is shown in Fig. 1. It should be noted that the second synchronous cycle shows a similar but less marked effect, presumably because cell synchrony is decreasing. This result clearly indicates that conjugation occurs primarily in the G_1 phase of a synchronous mixed population and that it is inhibited during the S phase.

We have substantiated this latter observation by using hydroxyurea to block DNA synthesis in synchronous cultures³. A population in which DNA synthesis was prevented maintained a high level of conjugation (70%) even though budding continued. As the inhibited cells passed through the block in DNA replication, the level of conjugation decreased to less than 10%. In other words, a blockage in DNA replication does

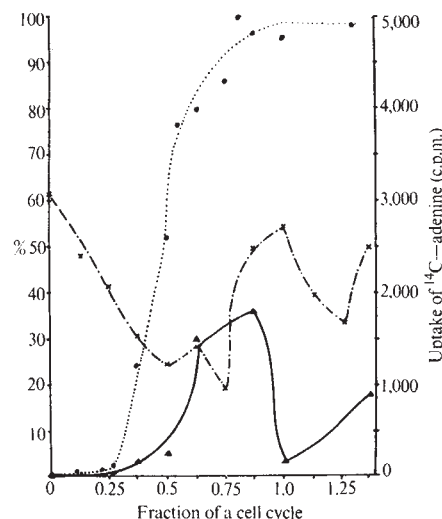


Fig. 1 The cell cycle and mating ability. About 10^8 cells of a log phase population containing approximately equal numbers of the two mating types were centrifuged in a 16 ml 0–30% sucrose gradient for 10 min at 300g at room temperature. The top fraction, about 2 ml containing roughly 10^8 unbudded cells, was inoculated into unlabelled complete medium and into a parallel culture that included $0.1 \mu\text{Ci ml}^{-1}$ ¹⁴C-adenine (¹⁴C-8-adenine, 54 mCi mmol⁻¹, International Chemical and Nuclear Corp.) in the complete medium. Samples were removed at intervals, centrifuged, washed in distilled water and transferred to non-nutrient agar then scored after 24 h incubation at 22° C for percentage conjugation. Aliquots were also withdrawn from the labelled culture, washed and hydrolysed with 0.2 ml N KOH for 12–16 h at room temperature. DNA was precipitated from the alkaline hydrolysate by adding excess 20% trichloroacetic acid and the precipitate was collected. The radioactivity was determined using a liquid scintillation detector. Samples of the growing cultures were also observed using acridine-orange fluorescence microscopy to determine the mitotic index. The budding index (percentage of cells with buds) was also determined, and it was noted that bud outgrowth was synchronous and was initiated shortly after the start of S phase. The generation time of the organism was approximately 8 h in this experiment. The terms G_1 , S and G_2 refer to the period from termination of mitosis to the beginning of DNA replication, the period of DNA synthesis and the time from completion of nuclear replication to the start of mitosis. ▲, Percentage mitosis; ●, DNA ¹⁴C-adenine; X, percentage conjugation.

not prevent budding, yet maintains the culture in a state of readiness for mating. Again cells entering S phase lost their ability to mate.

To test whether or not the two mating types were under similar control during the cell cycle we mated cells in synchronous stages of the cell cycle with cells of the opposite mating type in random stages of the cell cycle. The results of a typical experiment (Fig. 2) demonstrate that the cell cycle effect observed in mixed cultures is due solely to the refractoriness of mating type a_1 during the phases of the cell cycle beyond G_1 . Mating type a_2 maintains its readiness for copulation throughout the cell cycle. These cell cycle controls of each of the two mating type alleles are expressed in both the prototrophic original isolates¹ and amino acid auxotrophs derived from them by mutation and recombination. We have termed the behaviour of mating type a_1 a stringent cell cycle control while mating type a_2 has a relaxed cell cycle control. The terms stringent and relaxed do not refer to the influence of amino acid auxotrophy but to the cell cycle control of the expression of the mating type alleles. The experiments that we have completed so far are entirely consistent with a hypothesis that there is a sex message which is an mRNA molecule or molecules that is transcribed from the gene or genes governing sexual morphogenesis. The sex message is translated into proteins that organize and assemble the organelle for conjugation (the conjugation tube). The mechanism governing transcription of the sex message in sporidia at different stages in their cell cycle has provided a unique and revealing insight into the regulation of sexual morphogenesis determined by a relatively simple

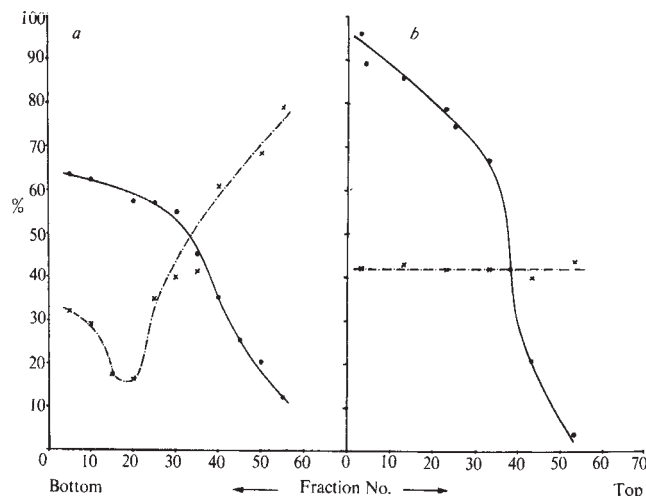


Fig. 2 The cell cycle regulation of mating ability is different in the two mating types. Log phase populations of either (a) a_1 or (b) a_2 haploids were separated using a 15–30% sucrose gradient in a Sorvall re-orienting zonal rotor. Cells were collected in fractions starting at the bottom of the gradient (these fractions were mainly large budded cells in G_2). The cells in different fractions were tested for their ability to mate with an equal number of cells from a log phase culture of opposite mating type (percentage conjugation (X)). The budding index (percentage of cells with buds) in each fraction was also determined (●).

mating type locus. The requirement that mating type a_1 undergo sexual morphogenesis during G_1 is reminiscent of the morphogenetic behaviour of most higher eukaryotes whose cells differentiate almost exclusively during G_1 . The mating allele a_1 has, like the chromosomes of higher organisms, a stringent cell cycle regulation over the morphogenetic activity that it affects. The cell bearing dominant allele a_2 enters sexual morphogenesis during most of the cell cycle, and so regulation of the activity of the allele is termed relaxed. A fuller elucidation of the molecular basis of the control difference between alleles a_1 and a_2 could enhance an understanding of the mechanisms that limit the differentiation of higher cells to the G_1 phase.

J. E. CUMMINS
A. W. DAY

Department of Plant Sciences,
University of Western Ontario,
London, Ontario N6A3K7

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- ¹ Day, A. W., and Jones, J. K., *Genet. Res.*, **2**, 63 (1968).
² Mitchison, J. M., and Vincent, W. S., *Nature*, **205**, 987 (1965).
³ Young, C. W., and Hodas, S., *Science*, **146**, 1172 (1964).

Temporal Allelic Interaction, a New Kind of Dominance

Ustilago violacea has a simple bipolar heterothallic mating system, with two alleles a_1 and a_2 of the mating-type locus. Haploid yeast-like cells of opposite mating type conjugate on a medium free of nutrients¹. Earlier reports from this laboratory using diploid, triploid and tetraploid cells showed that allele a_2 is dominant in log phase cultures (heterozygous cells conjugate with cells homozygous for a_1 but not cells homozygous for a_2). Heterozygous cells in stationary phase cultures failed to mate even though all the stationary phase homozygous cells mated actively. Consequently, the dominance of allele a_2 is termed age-related².

We can now explain age-related dominance in the light of recent experiments on the cell cycle control of sexual morphogenesis. We report elsewhere that in haploid cells allele a_1 can be induced to transcribe "sex message" only when the cell is in G_1 (a stringent cell cycle control) while its dominant allele a_2 can be induced during the entire cell cycle (a relaxed cell cycle

control)³. Furthermore, we have observed that as in most other organisms, stationary phase cultures of *U. violacea* are arrested in G_1 . Thus a hypothesis to explain age-related dominance became apparent. We argued that heterozygous diploids (a_1a_2) may retain the cell cycle controls of both alleles. In that event the stationary phase cells that are arrested in G_1 may produce sex message at both of the alleles and their products might effectively cancel out when produced simultaneously within the same cell. This would explain why stationary phase a_1a_2 cells mate with neither a_1 nor a_2 partners while stationary phase homozygous cells continue to mate with their appropriate partners.

If our premise that alleles a_1 and a_2 retain their unique cell cycle control features in the heterozygote is correct then in a log phase culture of the heterozygote those cells which were not in G_1 would produce a_2 sex message only because, as we have noted previously, the a_1 allele remains refractory to transcription during the later stages of the cell cycle. Allele a_2 would seem to be dominant, but only in those cells that were not in G_1 . Cells in G_1 , from a log phase population, would behave similarly to stationary phase cells in the sense that they would not mate with either partner.

We have confirmed the general features of this hypothesis by using two experimental approaches. First, we determined the ability of a_1a_2 cells to conjugate with a_1 partners during the synchronous growth that follows reinoculation of a stationary culture to fresh medium. DNA replication was followed in the culture using a parallel inoculum grown in the presence of ¹⁴C-adenine. As Fig. 1 shows, the synchronous population regains its ability to conjugate within 30 min of reinoculation and the percentage conjugation remains high throughout the subsequent S phase, through the G_2 phase and decreases rapidly during the following G_1 phase. These observations strongly support our hypothesis that the simultaneous activity of both mating type alleles produces a sexually neutral strain during the G_1 phase of the cell cycle while the innate repression of allele a_1 during the later parts of the cell cycle allows allele a_2 to be expressed. The residual activity during G_1 of the second cell cycle following reinoculation most likely results from a lack of complete synchrony, as many cells would not have completed their first cycle.

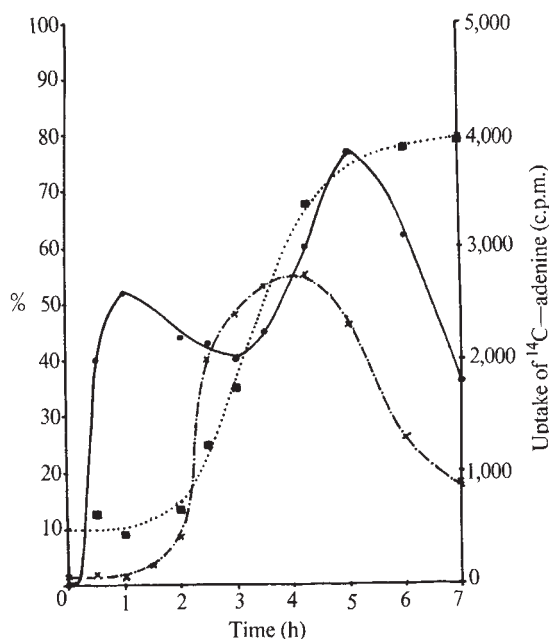


Fig. 1 The recovery of mating ability on outgrowth of a stationary phase heterozygous diploid. a_1a_2 cells were re-inoculated to complete medium at 0 h and samples were tested for ability to mate with an equal number of cells from a log phase a_1 culture (% conjugation (●)). Incorporation of ¹⁴C-adenine into DNA was followed in a parallel culture (■)³. The budding index was also determined (X).

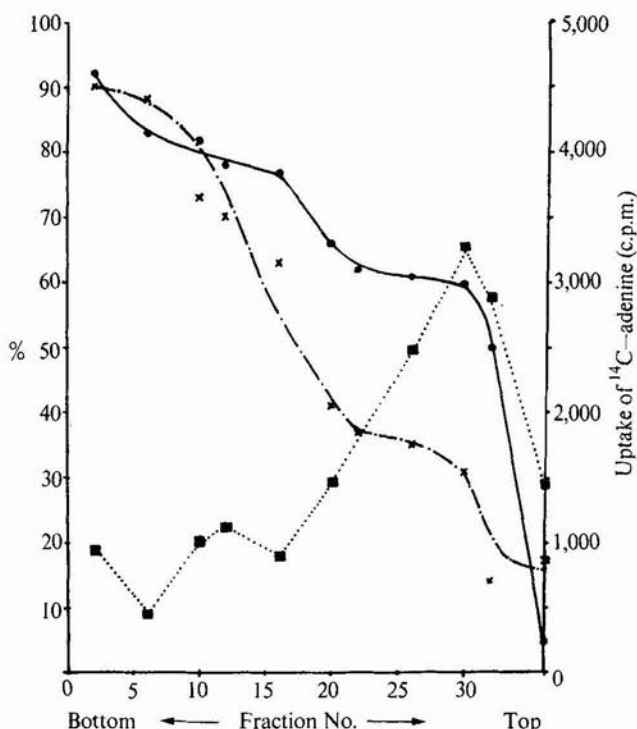


Fig. 2 The effect of the cell cycle on mating ability of a heterozygous diploid. A log phase a_1a_2 population was collected in fractions after sucrose gradient centrifugation. The fractions were tested for ability to mate with a stationary phase a_1 culture (% conjugation (●)). The budding index (X) and incorporation of ^{14}C -adenine into DNA in a one hour pulse (■) were also determined for different fractions. The incorporation of ^{14}C -adenine into DNA was determined as in ref. 3, but differs in the sense that the rate of adenine incorporation is being measured in the present experiment.

A second experiment, involving a log phase a_1a_2 population, provided strong confirmation of the initial observations on regulation of sexual expression in the heterozygous strain. In this experiment, fractions at different stages of the cell cycle were collected from the logarithmic population using a 15%–30% sucrose gradient in a re-orienting zonal rotor of the ultracentrifuge⁴. The G_2 cells which have almost terminated the budding cycle were mainly at the bottom of the sucrose gradient and most of the unbudded G_1 cells were at the top of the gradient, while the S phase cells with small buds were in the middle. Figure 2 shows the percentage conjugation of a_1a_2 cells in different fractions of the gradient together with the ability of these fractions to incorporate ^{14}C -adenine into DNA. The percentage conjugation was very high (about 90) in fractions that contain mainly G_2 cells and very low in fractions containing many G_1 cells. There is also indication that G_2 cells may be more competent to mate than S phase cells.

Both experiments described here show conclusively that a_1a_2 cells cannot mate while in the G_1 phase of the cell cycle, but that as the cell progresses through its cycle into the S and G_2 phases it acquires the ability to conjugate. The sexually competent cells behave as if the a_2 allele were dominant, therefore confirming the hypothesis suggested from work on the cell cycle control of separate alleles in haploid cells³. This genetic control system exemplifies a hitherto undetected mechanism of dominance, namely one based on different temporal properties of the alleles, such that the heterozygote has the combined temporal properties of the individual homozygotes.

In the mating system described here the combined effect of the two active alleles is to neutralize the expression of both. If, however, the unique temporal properties of the alleles of other loci are preserved in the heterozygous state and these alleles are additive, then a hybrid could be more active than either of its homozygous parents. We suggest that the type of

temporally determined allele behaviour reported here may perhaps be involved in heterosis, as a heterozygote may have a longer time period for transcription than either homozygote.

A. W. DAY
J. E. CUMMINS

Department of Plant Sciences,
University of Western Ontario,
London, Ontario N6A3K7

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¹ Day, A. W., and Jones, J. K., *Genet. Res.*, **2**, 63 (1968).

² Day, A. W., *Nature new Biol.*, **237**, 282 (1972).

³ Cummins, J. E., and Day, A. W., *Nature*, **245**, 259 (1973).

⁴ Wells, J. R., and James, T. W., *Expt Cell Res.*, **75**, 465 (1972).

Sex-associated Differential Fluorescence of Mudminnow Chromosomes and Spermatozoa

Most fishes have morphologically undifferentiated sex chromosomes and sex determination is believed to be under gene control, probably through the production of alternative enzymes¹. The idea that differentiation of fish sex chromosomes is usually relatively primitive has been supported by genetic experiments involving, for example, sex reversals and gynandromorphisms². Recently, however, sex chromosomes of the XX-XY, XX-XO, and WZ-ZZ types^{3–4}, as well as multiple sex chromosomes^{5,6}, have been documented in certain groups of fishes. Thus, fishes seem to display various cytological sex-determining mechanisms, some primitive, others elaborately specialized.

We believe that the differential fluorescence observed in morphologically identical chromosomes in a significant percentage of males of the mudminnow, *Umbra limi*, represents a "heterogenic" sex-determining mechanism that may be an early step in the evolution of heteromorphic sex chromosomes.

The distal end of the long arm of the human Y chromosome fluoresces when stained with quinacrine mustard⁷ or quinacrine dihydrochloride⁸, and this region can be detected in approximately 50% of human spermatozoa^{9–11}. We therefore investigated the possibility of identifying such fluorescing bodies (F bodies), which might be associated with sex determination, in the spermatozoa and chromosomes of fishes.

Male and female specimens of the central mudminnow, *U. limi* (Kirtland) were collected from two populations: Crane Brook, 1 mile north of Montezuma, Cayuga County, New York, and Duck Lake, at Spring Lake, Cayuga County, New York. Flame-dried chromosome preparations^{12,13} were made from testis, gill, fin, kidney and spleen tissues 5 h after the specimens had received a 1 ml intraperitoneal injection of 0.05% colchicine. For spermatozoa and meiotic preparations, ripening testes were removed, swollen for 20 min in distilled water, fixed for 5 min in 3:1 methanol-glacial acetic acid, and dabbed onto a microscope slide to release a slurry of cells which was then flame-dried. These slides were then stained with quinacrine dihydrochloride¹⁴ and viewed with a Leitz Orthoplan fluorescence microscope using a Ploem vertical illuminator and HB 200 type W4 high pressure mercury burner. BG 12 and BG 38 exciting filters were used with a combination of K 510 suppression filter and KP 490 interference blue filter.

The chromatin within the heads of spermatozoa fluoresced evenly, and 81–96% ($\bar{X}$ = 88%) of the spermatozoa from sixteen out of twenty males had a distinctive, much brighter F body (Fig. 1E, Table 1). In four out of twenty males, only 42–58% ($\bar{X}$ = 50%) of the spermatozoa exhibited an F body. Some of the spermatozoa exhibited two F bodies (Table 1), which may reflect one or more of the following possibilities. (1) The spermatozoa were diploid; (2) there was non-disjunc-

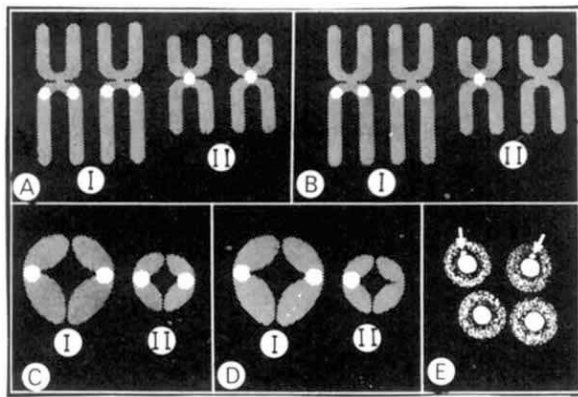


Fig. 1 Diagram showing position of F bodies (white circles) in fluorescing mitotic chromosomes of *U. limi* in 80% of males and 100% of females (A) and 20% of males (B). Diagram showing position of F bodies in fluorescing meiotic chromosomes in 80% of males (C) and 20% of males (D). Diagram showing position of F bodies (arrowed) in spermatozoa (E). Although not shown in the diagram, the F bodies in chromosome pair II were much brighter than in pair I.

tion of chromosome pair II (Fig. 1C) at meiosis; (3) a centric fission-fusion mechanism is operating in chromosome pair II of some males resulting in the concentration of fluorescing material in one chromosome; or (4) some background fluorescence was shining through.

The diploid karyotype of *U. limi*¹⁵ consisted of twenty-two chromosomes, eighteen metacentrics and four submetacentrics. No heteromorphic sex chromosomes could be distinguished. All the chromosomes exhibited an even, moderate fluorescence except for the much brighter F bodies situated close to or at the centromeres in two pairs of chromosomes (Fig. 2). No differentially fluorescent banding patterns were evident as in humans and other mammals.

The sixteen males exhibiting F bodies in 88% of the sperm also had prominent F bodies on four metacentric chromosomes (Fig. 1A; Fig. 2). Two apparently homologous chromosomes, here designated pair I, each had an F body on each chromatid just below the centromere while another pair of smaller metacentrics, pair II, each had a fluorescing F body (actually three fused F bodies) at the region of the centromere (Fig. 1A). In these same males, chromosome pairs I and II exhibited F bodies at meiosis but the fluorescence in the two F bodies in each chromosome of pair I appeared fused into one F body because of their highly contracted state and proximity (Fig. 1C).

The four males exhibiting F bodies in 50% of the spermatozoa had only three mitotic chromosomes exhibiting F bodies. Both members of pair I and one member only of pair II had F bodies (Fig. 1B). Likewise, at meiosis, both members of pair I had an F body, but only one member of pair II had this structure (Fig. 1D). The F body seen in one member of pair II exhibited a much greater fluorescent intensity than F bodies in pair I chromosomes. Thus, the 50% frequency of

F bodies in spermatozoa of these four males was exactly the expectation for segregation of pair II chromosomes at meiosis.

All twenty females had F bodies on mitotic chromosomes of pairs I and II in the exact location as 80% of the males (Fig. 1A). Thus the females and 80% of males were "homogenic" for the F body in chromosome pairs I and II, while 20% of males were "heterogenic" for the F body in pair II.

The sex-associated differential fluorescence in chromosomes which are not morphologically differentiated may reflect differences in DNA composition (high G-C or A-T content) or differences in gene activity as a function of DNA-associated non-histone protein¹⁶⁻¹⁸. In any event, the net result would be sex-associated differential gene action.

The fact that not all males were heteromorphic for the F body warrants further research. Three hypotheses seem applicable. (1) There is a polymorphism of the nuclear non-histone protein as a result of a male hormone-chromosome interaction. This occurs in all males, but is grossly visible by quinacrine fluorescence only in some. Thus, degrees of hormone-chromosome interaction may vary¹⁹. In this connexion it is interesting to note the non-fluorescence in the Y chromosome and spermatozoa of a human male with primary infertility and a low sperm count²⁰. (2) A centric fission-fusion mechanism is operating in chromosome pair II of some males resulting in a concentration of fluorescing material in one chromosome. If the exchange is reciprocal, pairing at meiosis is normal. (3) The homogenic males could be in the beginning stages of sex reversal and are already expressing cytological "femaleness" in their fluorescence patterns. Both protandrous and protogynous hermaphroditism have been reported in fishes²¹, and there is some evidence that smaller and younger specimens of *U. limi* are predominantly males, while larger and older individuals are mostly females²². This evidence, although circumstantial, suggests that some protandrous hermaphroditism may be operating in some populations of *Umbra limi*.

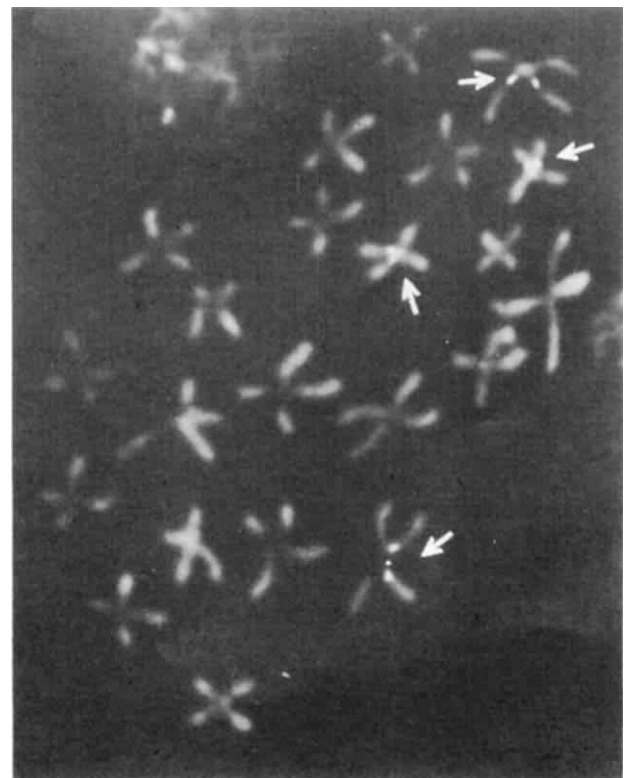


Fig. 2 Mitotic metaphase spread of fluorescing chromosomes from gill epithelium of "homogenic" male of *U. limi*. White arrows point to chromosomes exhibiting F bodies. (Longest chromosome is about 9 μ m long.)

Table 1 Comparison of Percentage of Spermatozoa Exhibiting 0, 1 or 2 F Bodies in Males from Two Populations of *Umbra limi*

No. of specimens	% of spermatozoa with 0, 1, or 2 F bodies*			No. of observations
	0	1	2	
Crane Brook population				
7	7.0 $\pm$ 3.5	89.4 $\pm$ 3.2	3.6 $\pm$ 2.8	700
3	50.4 $\pm$ 8.4	46.4 $\pm$ 6.2	3.2 $\pm$ 0	1,100
Duck Lake population				
9	4.1 $\pm$ 3.1	86.7 $\pm$ 5.2	9.2 $\pm$ 5.4	2,000
1	42.5 $\pm$ 0	57.5 $\pm$ 0	0.0	200

* Mean $\pm$ s.d.

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W. MIKE HOWELL

Section of Ecology and Systematics,
Langmuir Laboratory,
Cornell University,
Ithaca, New York 14850

STEPHEN E. BLOOM

Department of Poultry Science,
Cornell University,
Ithaca, New York 14850

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- ¹ Mittwoch, U., *Sex Chromosomes* (Academic Press, New York, 1967).
- ² Chen, T. R., thesis, Yale Univ. (1967).
- ³ Chen, T. R., *Postilla*, 130, 1 (1969).
- ⁴ Chen, T. R., and Ebeling, A. W., *Chromosoma*, 18, 88 (1966).
- ⁵ Uyeno, T., and Miller, R. R., *Nature*, 231, 452 (1971).
- ⁶ Uyeno, T., and Miller, R. R., *Experientia*, 28, 223 (1972).
- ⁷ Zech, L., *Expl. Cell Res.*, 58, 463 (1969).
- ⁸ Vosa, C. G., *Chromosoma*, 30, 366 (1970).
- ⁹ Barlow, P., and Vosa, C. G., *Nature*, 226, 961 (1970).
- ¹⁰ Pearson, P. L., Bobrow, M., and Vosa, C. G., *Nature*, 226, 78 (1970).
- ¹¹ Sumner, A. T., Robinson, J. A., and Evans, H. J., *Nature new Biol.*, 229, 231 (1971).
- ¹² Denton, T. E., and Howell, W. M., *Copeia*, 2, 392 (1969).
- ¹³ Howell, W. M., *Copeia*, 1, 191 (1972).
- ¹⁴ Breg, W. R., *Stain Technol.*, 47, 89 (1972).
- ¹⁵ Foley, J. O., *Biol. Bull.*, 50, 117 (1926).
- ¹⁶ Comings, D. E., *Expl. Cell Res.*, 67, 441 (1971).
- ¹⁷ Caspersen, T., Farber, S., Foley, G. E., Kudynowski, J., Modest, E. J., Simonsson, E., Wagh, U., and Zech, L., *Expl. Cell Res.*, 49, 219 (1968).
- ¹⁸ Ellison, J. R., and Barr, H. J., *Chromosoma*, 36, 375 (1972).
- ¹⁹ Madhabananda, S., and Stumpf, W. E., *Science*, 179, 389 (1973).
- ²⁰ Retief, A. E., and Van Mierkerk, W. A., *Lancet*, ii, 270 (1971).
- ²¹ Atz, J. W., in *Intersexuality in Vertebrates Including Man* (edit. by Armstrong, C. N., and Marshall, A. J.), 145 (Academic Press, London, 1964).
- ²² Applegate, V. C., *Copeia*, 2, 92 (1943).

Low and High Affinity Antibodies Can Alternate during the Immune Response

ANTIBODY affinity usually varies during the course of the immune response. This information has been acquired by studying antibodies present in the serum (for review see ref. 1), released by single cells in semi-solid media²⁻¹⁰ or, more recently, synthesized *in vitro* and released into liquid medium by lymph node microfragments^{11,12}.

Early reports¹³⁻¹⁶ suggested a steady increase in antibody affinity up to a plateau but this is not always the case. Recent work has demonstrated variations in antibody affinity with time after immunization which parallel the modifications in antibody titres¹⁷, and a fall in the association constant by the end of the response⁷. Moreover, it has been shown that antibodies with low affinity persist for a long time, even after high affinity populations have declined^{18,19}. In some cases, antibodies with a distinctive affinity, functionally homogeneous or very restricted, appear very early and remain unchanged over long periods²⁰⁻²². Controversial data also exist with regard to variations of antibody heterogeneity in terms of affinity. Some workers have found a progressive increase in heterogeneity^{9,13} while others have reported the reverse, or more complicated patterns^{4-6,12,23-27}. It would seem then that variations in antibody affinity, corresponding to changes in antibody populations which in turn indicate modifications at the level of antibody-forming clones, are complicated and do not follow a constant pattern.

Part of this complexity may arise because in some cases the

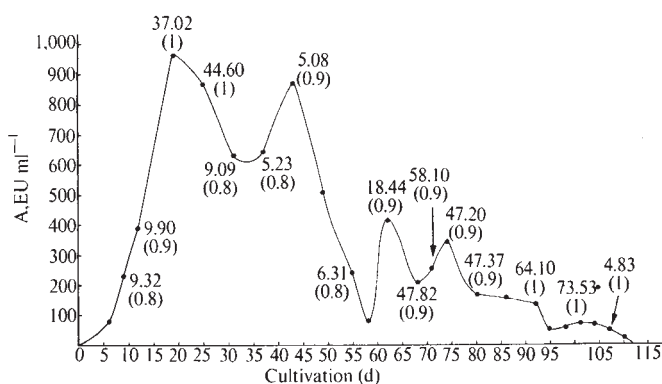


Fig. 1 Profile of the anti- β -D-galactosidase activating-antibody titres (active enzyme units (A, EU) ml^{-1}) during the entire course of an immune response *in vitro*. The culture tube contained five lymph node microfragments ($\sim 5 \times 10^4$ cells per fragment) from a rabbit immunized with 1 mg of the enzyme (in complete Freund's adjuvant, one injection, in the foot-pad) 1 yr before starting the culture. Each microfragment was challenged at zero time with 5 μg of enzyme for 30 min, at 37° C. Four consecutive antibody-titre cycles can be distinguished, taking about 1 month each. Figures close to the curve indicate the association constant (K_0 in $1 \text{ mol}^{-1} \times 10^5$) of the activating antibodies at the corresponding time of cultivation. Figures between parentheses indicate the heterogeneity index (α).

data are fragmentary. Serum and/or cell samples may have been taken too far apart, precluding the construction of a profile of the time-course variations in antibody affinity after immunization. Usually sera from various bleedings have been pooled and, from these, antibodies have been purified. Both manipulations may have led to intermixing and artefactual selection of antibody molecules. Moreover, in most studies antibodies have been induced and synthesized *in vivo*, in the whole animal. In this situation antigenic stimulation and antibody production are subjected to several regulatory and interfering factors which cannot be controlled or avoided. Furthermore, antibodies reaching the serum from various antibody-forming clones make a complex mixture which renders difficult the identification and characterization of antibody populations.

As accurate data on the sequential appearance of different antibody populations are essential to the understanding of the mechanism of clonal dynamics after antigenic challenge, we have developed an *in vitro* system that makes possible the study of this process under relatively simpler and better controlled conditions (refs 12, 28 and 29). The system consists of a combination of a microculture method^{12,28} with the use of β -D-galactosidase (*E. coli*) as an antigen³⁰⁻³². This makes it possible for antibody measurements using very small samples to be made. Antibodies elicited and synthesized *in vitro* by lymph node microfragments can be serially studied, every 2 to 5 d for several months, avoiding the necessity of pools and purifications. The methods have been published elsewhere (refs 11, 12, 28 and 29).

In previous studies we analysed microcultures containing small numbers of cells (10^4 to 10^5) and the antibody response could be followed for up to 40 d. We observed either a progressive rise in antibody affinity with time of cultivation, or no modifications of the values found in very early samples¹². Here we describe another pattern in which affinity variations follow an undulating profile. This pattern was observed only in multifragment cultures producing a long lasting antibody response (more than 100 d), that is, in four out of ten cultures reported here.

In Fig. 1, four consecutive antibody-titre cycles can be distinguished, taking about one month each. This pattern was confirmed in three separate determinations. Early antibodies, those present in the supernatant of the cultures between

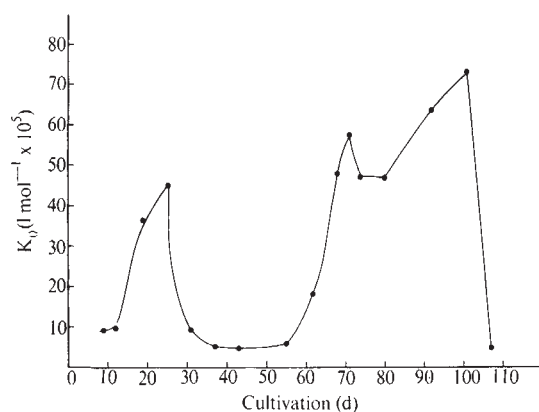


Fig. 2 Sequential changes in the association constant of the anti- β -D-galactosidase activating-antibodies during the immune response *in vitro* shown in Fig. 1.

5 and 10 d, are of low affinity and rather heterogeneous. Antibody affinity changes cyclically (Fig. 2) in a way that for each rise and fall in antibody titre, there is a distinctive affinity correspondence. Within each antibody-titre cycle the association constant tends to rise, but a decline occurs by the end of the cycles. Considering the entire course of the response the sequential modifications in antibody affinity do not proceed uninterruptedly towards higher values, but rather low and high affinity antibodies predominate alternately (Fig. 2). Very late antibodies (the latest that can be measured by the end of the response) are of low affinity. The degree of heterogeneity increases where two antibody-titre cycles join, which indicates, at this point, an overlapping of antibody populations with different affinities.

The oscillations in antibody titres resemble the variations observed in mice immunized with the somatic antigen of *E. coli*³³ and can be seen in the example shown in Fig. 1, probably because the sequential replacement of antibody populations is simple enough to be noticed. This may be because of stimulation of very few clones, one after the other, with little overlapping. The phenomenon could be explained by an alternating predominance of antibody-forming clones; when the dominant clone decays to a low level of antibody synthesis another one can be stimulated³⁴ and, in turn, take over the response.

The continuous replacement of antibody-forming clones underlying the consecutive changes in antibody affinity is, then, more complex than envisioned in the past. These changes cannot be explained simply by the concept of cell selection by antigen¹ in its simplest version. This postulates that decaying amounts of antigen selectively stimulate antibody-forming cell precursors bearing surface, antibody-like receptors with the higher affinity, and implies a constant rise in the association strength of the secreted antibodies. Although this hypothesis may be essentially correct^{2-5,35} it seems at present to be an over-simplification^{19,36}.

Other mechanisms may also be involved in determining the dynamics of the clonal replacement during the immune response. In this respect cell to cell collaboration may play some role, for example by helping antibody-forming cell precursors endowed with receptors with relatively lower affinity to be triggered, and even to take over the response. Whatever the mechanism may be, the alternation of low and high affinity antibody-forming clones would have a great selective advantage in promoting an enlargement of the memory potential and avoiding its restriction to high affinity memory cells. In this manner the organism would acquire the capability to react adequately against a wide range of antigen doses on subsequent aggressions. This idea is supported by the fact that late after immunization a broad spectrum of memory cells co-exist in terms of affinity with a restricted serum antibody population of high affinity²⁵.

The biological significance of late antibodies with low affinity remains to be established. Hypothetically, one may think that they are molecules released by memory cells, or a T cell product³⁷ which, on interaction with antigen, form aggregates that would facilitate stimulation of antibody-forming cell precursors^{37,38}.

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ALBERTO J. L. MACARIO
EVERLY CONWAY DE MACARIO

Laboratory of Cell Biology,
Via Romagnosi 18/A, 00196 Rome

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- ¹ Siskind, G. W., and Benacerraf, B., *Adv. Immun.*, **10**, 1 (1969).
- ² Andersson, B., *J. expl. Med.*, **132**, 77 (1970).
- ³ Andersson, B., *J. expl. Med.*, **135**, 312 (1972).
- ⁴ Claflin, L., and Merchant, B., *Cell. Immun.*, **5**, 209 (1972).
- ⁵ Claflin, L., Merchant, B., and Inman, J., *J. Immun.*, **110**, 241 (1973).
- ⁶ Claflin, L., and Merchant, B., *J. Immun.*, **110**, 252 (1973).
- ⁷ Doria, G., Schiaffini, G., Garavini, M., and Mancini, C., *J. Immun.*, **109**, 1245 (1972).
- ⁸ Huchet, R., and Feldmann, M., *Eur. J. Immun.*, **3**, 49 (1973).
- ⁹ Miller, G. W., and Segre, D., *J. Immun.*, **109**, 74 (1972).
- ¹⁰ Wu, C. Y., and Cinader, B., *Eur. J. Immun.*, **2**, 398 (1972).
- ¹¹ Macario, A. J. L., Conway de Macario, E., Franceschi, C., and Celada, F., in *Advances in Experimental Medicine and Biology* (edit. by Lindahl-Kiessling, K., Alm, G., and Hanna, jun., M. G.), **12**, 365 (Plenum Press, New York and London, 1971).
- ¹² Macario, A. J. L., Conway de Macario, E., Franceschi, C., and Celada, F., *J. expl. Med.*, **136**, 353 (1972).
- ¹³ Eisen, H. N., and Siskind, G. W., *Biochemistry*, **3**, 996 (1964).
- ¹⁴ Steiner, L. A., and Eisen, H. N., *Bact. Rev.*, **30**, 383 (1966).
- ¹⁵ Steiner, L. A., and Eisen, H. N., *J. expl. Med.*, **126**, 1161 (1967).
- ¹⁶ Goidl, E. A., Paul, W. E., Siskind, G. W., and Benacerraf, B., *J. Immun.*, **100**, 371 (1968).
- ¹⁷ Urbain, J., van Acker, A., de Vos-Cloetens, C., and Urbain-Vasanten, G., *Immunochemistry*, **9**, 121 (1972).
- ¹⁸ Werblin, T. P., and Siskind, G. W., *Immunochemistry*, **9**, 978 (1972).
- ¹⁹ Werblin, T. P., Kim, Y. T., Quagliata, F., and Siskind, G. W., *Immunology*, **24**, 477 (1973).
- ²⁰ Gopalakrishnan, P. V., Hughes, W. S., Kim, Y., and Karush, F., *Immunochemistry*, **10**, 191 (1973).
- ²¹ Haber, E., and Stone, M., *Israel J. med. Sci.*, **5**, 332 (1969).
- ²² Montgomery, P. C., Rokey, J. H., and Williamson, A. R., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 228 (1972).
- ²³ Kitagawa, M., Grossberg, A. L., Yagi, Y., and Pressman, D., *Immunochemistry*, **4**, 157 (1967).
- ²⁴ Roholt, O. A., Seon, B. K., and Pressman, D., *Immunochemistry*, **7**, 329 (1970).
- ²⁵ Macario, A. J. L., Conway de Macario, E., and Celada, F., *Nature new Biol.*, **241**, 22 (1973).
- ²⁶ Jaton, J. C., Waterfield, M. D., Margolies, M. M., Bloch, K. J., and Haber, E., *Biochemistry*, **10**, 1583 (1971).
- ²⁷ Kimball, J. W., *Immunochemistry*, **9**, 1169 (1972).
- ²⁸ Macario, A. J. L., Conway de Macario, E., and Celada, F., *Immunology*, **24**, 237 (1973).
- ²⁹ Celada, F., Macario, A. J. L., and Conway de Macario, E., *Immunochemistry* (in the press).
- ³⁰ Rotman, B., and Celada, F., *Proc. natn. Acad. Sci. U.S.A.*, **60**, 660 (1968).
- ³¹ Celada, F., Ellis, J., Bodlund, K., and Rotman, B., *J. expl. Med.*, **134**, 751 (1971).
- ³² Celada, F., Strom, R., and Bodlund, K., in *The Lactose Operon* (edit. by Beckwith, J. R., and Zipser, D.), 291 (Cold Spring Harbor Laboratories Press, Cold Spring Harbor, N.Y., 1970).
- ³³ Britton, S., Wepsic, T., and Moller, G., *Immunology*, **14**, 491 (1968).
- ³⁴ Askonas, B. A., and Williamson, A. R., *Nature*, **238**, 339 (1972).
- ³⁵ Davie, J. M., and Paul, W. E., *J. expl. Med.*, **135**, 660 (1972).
- ³⁶ Segre, D., and Segre, M. (in the press).
- ³⁷ Feldmann, M., and Basten, A., *Eur. J. Immun.*, **2**, 213 (1972).
- ³⁸ Feldmann, M., and Nossal, G. J. V., *Q. Rev. Biol.*, **47**, 269 (1972).

Collagen of Marfan Syndrome is Abnormally Soluble

MARFAN syndrome, which is inherited as an autosomal dominant trait with variable expressivity, results from a diffuse or generalized disorder of connective tissues. It

is not known, however, whether the primary defect resides in elastic fibres, collagen or some other component of connective tissue¹. When patients die of the disorder it is usually because of abnormality of the connective tissues of the large vessels leaving the heart². We wish to present evidence that collagen produced by fibroblasts cultured from individuals with Marfan syndrome is more soluble than normal in solvents used to extract it. Such abnormality was first proposed by Macek *et al.*³.

We used cell cultures which had undergone between five and twenty population doublings after primary culture. Some of the cultures had been stored in liquid nitrogen for periods up to 7 yr. Cells were maintained in Dulbecco and Vogt's medium⁴ supplemented with 15% foetal bovine serum and when experiments were in progress this nutrient medium also contained 25 $\mu\text{g ml}^{-1}$ of sodium ascorbate to promote synthesis of collagen⁵. Replicate, 15 $\times$ 60 mm culture dishes were inoculated with 2–3 $\times$ 10⁵ cells in 4 ml of nutrient medium and allowed to grow for 14–18 d with changes of medium every 3 or 4 d. These cell layers which had remained at confluency for at least a week were then scraped into 2.5 ml of either 1.0 M NaCl buffered to pH 7.5 with 0.05 M Tris, or 0.5 M acetic acid. After transfer to small tubes with 'Teflon'-lined caps the collagen was extracted by shaking gently at 4° C for 24 h. After centrifugation the supernatant and precipitate were each hydrolysed in 5 ml of 6 N HCl at 105° C for 15 h and the amount of hydroxyproline determined⁶.

Total hydroxyproline and percentage extracted by neutral salt or acetic acid are recorded in Table 1. In the first series of experiments the contents of one 60 mm culture dish were assayed; in the second two dishes were combined and assayed. Other differences in methodology between the first and second series should be noted. In the first, use of a gyrorotatory shaker probably accounts for differences in the amount of collagen extracted in separate experiments since the vigour of extraction varied. In the second series, greater reproducibility but more vigour of extraction was obtained by a reciprocal shaker with a 1.25 inch stroke at 60 cycles per min (to and fro=one cycle). In spite of procedural variations, in each experiment a larger proportion of total collagen was extracted by acetic acid from six different Marfan cultures than

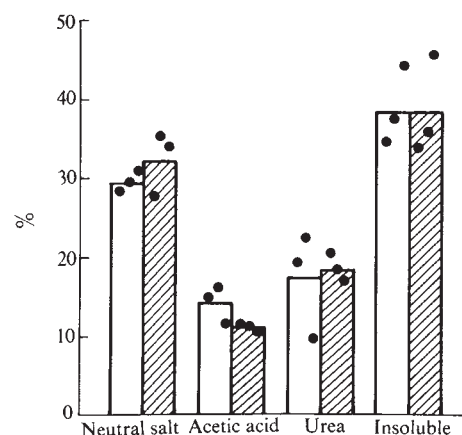


Fig. 1 Solubility of recently synthesized control (□, 125) and Marfan (▨, 341) collagens. Replicate control and Marfan cultures were exposed for 8 h to ³H-proline (1.0 $\mu\text{Ci ml}^{-1}$, 519 mCi mmol⁻¹) after 18 d of growth. The cell layers were then extracted sequentially by 2.5 ml of 1.0 M NaCl buffered to pH 7.5, 0.5 M acetic acid, and 8.0 M urea. Radioactivity in hydroxyproline of hydrolysed protein in the extracts and residue was then determined. The results were plotted as percentages of the total radioactivity in hydroxyproline for each replicate culture.

from controls. This difference varies from as little as 1.7 times as much for Marfan cultures to as great as 6.2 times as much, and averages 2.8 times for the first experiments and 2.4 times for the second series of experiments. There was no consistent difference between control and Marfan cultures in total amount of collagen present, nor did variation in amount of collagen produced affect the greater extraction of Marfan collagen by acetic acid. We postulate that a molecular defect in Marfan collagen accounts for its unusual solubility.

It is well recognized that there is a progressive increase in degree of crosslinking of collagen with time after synthesis and fibril formation^{7,8} which renders it progressively more insoluble. We therefore devised an experiment to compare the solubility of newly synthesized and older collagen of control and Marfan cultures. After 18 d of growth the cell layers of a series of replicate cultures

Table 1 Hydroxyproline Extracted from Cell Layers of Seven Control and Six Marfan Cultures

Culture No.	Control			Culture No.	Marfan		
	Total hydroxyproline (nmol)	Percentage extracted in:			Total hydroxyproline (nmol)	Percentage extracted in:	
		Neutral salt	Acetic acid			Neutral salt	Acetic acid
First series of experiments							
135	50	18.4	17.8	252	38	16.0	42.4
125	115	5.4	8.2	252	176	6.1	17.2
135	227	7.2	9.8	343	29	11.6	60.7
350	12	5.0	8.7	344	142	4.4	25.9
				345	26	7.3	29.0
309	40	9.0	13.9	346	35	8.3	23.0
Mean	89	9.0	11.7		74	9.0	33.0
Second series of experiments							
351	230	8.7	23.0	341	219	8.1	38.4
135	80	6.5	17.8	341	223	15.1	60.3
357	331	12.4	21.4	252	291	19.0	50.9
357	257	—	23.7	252	347	—	70.6
357	722	—	16.7	252	244	—	38.4
354	482	—	20.3	345	142	—	40.2
Mean	350	9.2	20.5		244	14.1	49.8

Each row of figures represents a separate experiment, and each number represents the mean of two or more determinations.

Two Marfan cultures (252 and 341) were established in this laboratory; one (343) was donated by Dr Arthur Falek, Georgia Mental Health Institute, Atlanta, Georgia; and the three others were obtained from the American Type Culture Collection Cell Repository, Rockville, Maryland. Our numbers 344, 345 and 346 correspond respectively to CRL 1128, CRL 1173 and CRL 1174. All individuals had established clinical diagnoses of Marfan syndrome. Two were from the same family (345 and 346). The source of the primary explants was skin. The non-Marfan cultures were all established in this laboratory in the Section of Medical Genetics. Explant sources were foetal skin (135), infant foreskin (125, 354 and 357), autopsy thymus (309), and adult skin (350 and 351).

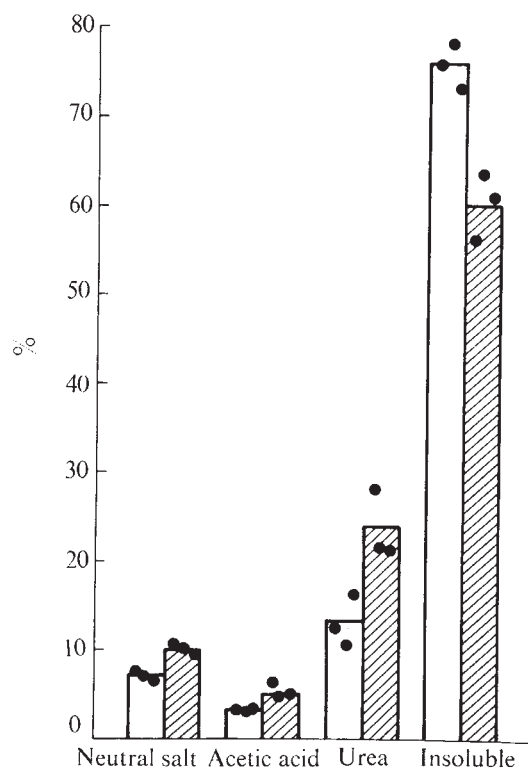


Fig. 2 Solubility of previously synthesized control (□, 125) and Marfan (▨, 341) collagen. Replicate cultures were exposed to ^3H -proline for 8 h on the fourteenth day of growth, fresh unlabelled medium applied and the cell layers extracted sequentially with neutral salt, acetic acid and urea 4 d after labelling as indicated in the legend to Fig. 1.

were subjected to sequential extraction first by neutral salt, then by acetic acid and finally by urea. Some of these cultures had been exposed for 8 h to radioactive proline 4 d earlier, while others were exposed to the isotope for the immediately preceding 8 h. The amount of radioactivity was determined in hydroxyproline of hydrolysed protein in the different extracts⁹. Using this specific labelling procedure newly synthesized collagen (Fig. 1) could be distinguished from older collagen synthesized 4 d earlier (Fig. 2). There was no consistent difference in solubility of collagen synthesized recently by control and Marfan cultures (Fig. 1) in any of the three solubilizing agents, and on average the same percentage remained insoluble. Consistent differences were found, however, in solubility of collagen produced 4 d earlier by control and Marfan cultures (Fig. 2). A greater proportion of Marfan collagen was extracted into each of the three solubilizing agents and less remained insoluble. Thus, the solubility properties of newly synthesized Marfan collagen differed little from normal, but after the formation of fibres, Marfan collagen failed to become insoluble to the same extent as did normal collagen. A second experiment gave similar results. Newly synthesized Marfan collagen was 1.2 times more soluble in all solvents than the control (compared with equivalent solubility in the first experiment), whereas older Marfan collagen was 2.5 times more soluble than control (compared with 1.7 times in the first experiment). Of course an abnormality could be present in newly synthesized Marfan collagen which is not expressed in its solubility properties until the fibres begin to mature.

The results of these experiments have not identified the molecular defect making Marfan collagen more soluble. Although we favour the hypothesis that a molecular defect is present in Marfan collagen which interferes with subsequent formation or stabilization of some of the crosslinks

between molecules in collagen fibres, other hypotheses could also explain the results reported here. An abnormality in proportion of the different kinds of alpha chains, three of which comprise a single collagen molecule, could also result in fibres that are partly abnormal and more susceptible to solubilization. It is even conceivable that the abnormal solubility of Marfan collagen is secondary to a molecular defect in some other component of connective tissue, although there is no firm basis in connective tissue biochemistry for this hypothesis.

The finding of abnormal solubility of Marfan collagen is important for at least two reasons. First, it may lead to clarification of the molecular defect in this disorder, and second, it may provide a basis for early diagnosis (possibly antenatally) on cultured cells, a problem which has assumed greater importance since the suggestion that prophylactic treatment with agents to reduce myocardial contractility (reserpine or propranolol) may delay or prevent the lethal effects of the disease in great vessels leaving the heart².

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ROBERT E. PRIEST
JESSIE F. MOINUDDIN

Department of Pathology,
Emory University,
Atlanta, Georgia 30322

JEAN H. PRIEST

Departments of Pediatrics and Pathology,
Emory University,
Atlanta, Georgia 30322

Received May 10, 1973.

- McKusick, V. A., *Heritable Disorders of Connective Tissue* (Mosby, St. Louis, 1966).
- Murdoch, J. L., Walker, B. A., Halpern, B. L., Kuama, J. W., and McKusick, V. A., *New Engl. J. Med.*, **286**, 804 (1972).
- Macek, M., Hurych, J., Chvapil, M., and Kadlecova, V., *Human-genetik*, **3**, 87 (1966).
- Priest, J. H., *Cytogenetics*, 172 (Lea and Febiger, Philadelphia, 1969).
- Priest, R. E., and Bublitz, C., *Lab. Invest.*, **17**, 371 (1967).
- Prockop, D. J., and Udenfriend, S., *Anal. Biochem.*, **1**, 228 (1960).
- Tanzer, M. L., and Mechanic, G., *Biochem. biophys. Res. Commun.*, **32**, 885 (1968).
- Mechanic, G., *Israel J. med. Sci.*, **7**, 458 (1971).
- Juwa, K., and Prockop, D. J., *Anal. Biochem.*, **15**, 77 (1966).

Ethanol Oxidation by Human Erythrocytes

ANALYTICAL studies, recently reported on the stability of ethanol in stored human blood, unexpectedly showed that human erythrocytes could oxidize ethanol to acetaldehyde¹. The mechanism^{2,3} was linked with the oxyhaemoglobin-methaemoglobin redox system⁴. I consider here the implications of blood ethanol oxidation in two relevant areas, namely, the stabilization of ethanol in blood samples taken from motorists who fail a breath test and also the possibility of ethanol metabolism by erythrocytes *in vivo*.

We were originally interested in the stability of ethanol in blood samples obtained as a result of current legislation for the control of the drinking driver. The appropriate legislation in this country is the Road Safety Act 1967, now consolidated in the Road Traffic Act 1972, which makes it an offence for the drinking driver to exceed 80 mg of ethanol per 100 ml of blood (80 mg%).

No increasing blood ethanol concentration was found in storage experiments. The consistent losses of ethanol in these samples from live individuals contrast strongly with the ethanol

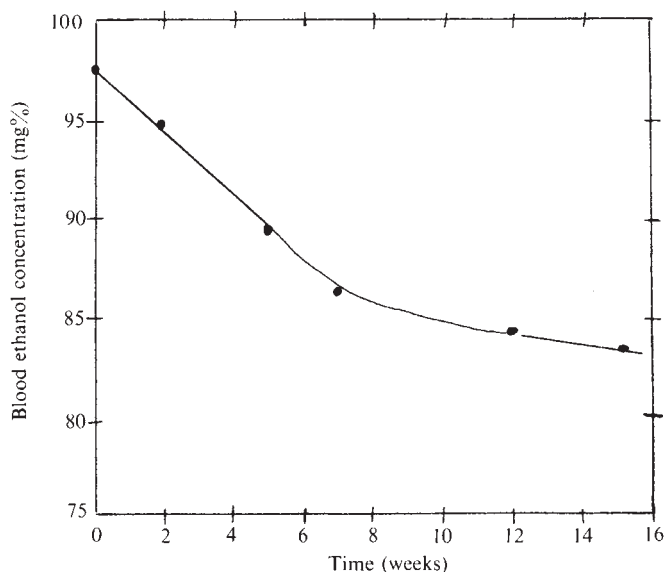


Fig. 1 Ethanol concentration against time for a blood sample at room temperature.

increases found by others in blood samples taken at autopsy^{5,6}. Ethanol losses in blood samples from live individuals had previously been observed⁷⁻⁹, but there was no satisfactory explanation for these and consequently no recommendations for an effective means of stabilization.

Scientific problems have been encountered with a small proportion of prosecutions under the Road Safety Act when cases have come to court¹⁰. A common feature in such cases has been a long period of storage of the defendant's sample at room temperature before analysis and in my experience the analyst engaged by the defence has found a significantly lower ethanol level than the forensic science laboratory which earlier provided the official analysis.

Three requirements must be satisfied before stable samples can be obtained from live individuals. The blood containers have to be effectively sealed, a preservative such as 1% sodium fluoride added to prevent microbial growth, and the ethanol oxidation mechanism has to be blocked with an inhibitor, sodium fluoride being ineffective in this respect. For samples at present collected in connexion with the Road Traffic Act the third condition is not satisfied and therefore I suggest that ethanol oxidation by erythrocytes has been a major source of inter-laboratory discrepancies.

The ethanol oxidized in whole blood plotted against time at a typical ambient temperature of approximately 20° C is shown in Fig. 1. The shape of this graph is independent of the initial ethanol concentration, and shows clearly how a sample which is originally well above the prescribed limit can fall, within a normal storage period for defence samples of a few weeks¹⁰, to a value below it, thus causing a difficult problem for the courts to resolve. As the standard deviation for blood ethanol determinations by gas chromatography¹¹ near the prescribed limit is currently below ± 2 mg% the resultant discrepancy is likely to be statistically significant.

Previous experience had shown that compounds which

destroyed oxyhaemoglobin inhibited ethanol oxidation in blood samples. Here I report the use of four inhibitors at concentrations of 0.5% w/v which can each stabilize ethanol in blood, dithionite and bisulphite because they reduce oxyhaemoglobin to haemoglobin, and nitrite and azide which cause oxidation of oxyhaemoglobin to methaemoglobin. Table 1 shows the change in ethanol concentration for a normal blood sample compared with the loss for four inhibited samples during 16 weeks at approximately 20° C. The inhibited samples do not show any significant change in ethanol concentration. Although further details of this work will be reported later, understanding of the oxidation mechanism has allowed stabilization of ethanol in blood to be achieved, even over long periods of storage at ambient temperatures.

The main site of ethanol metabolism *in vivo* is the soluble cytoplasm of liver cells although metabolism may also occur to some extent in several other organs including the brain¹². The dominant enzyme is alcohol dehydrogenase with nicotinamide adenine dinucleotide as the hydrogen acceptor. Ethanol may also be oxidized by the peroxidase-xanthine oxidase-catalase system or other oxidases¹³, the mixed function enzyme¹⁴ and also by the microsomal ethanol oxidizing system¹⁵.

The initial rate of ethanol oxidation *in vitro* was determined for the whole blood of ten individuals at 37° C and the result was 0.23 ± 0.06 mg% per h. Assuming that a similar rate occurred *in vivo* and the total blood volume was 5 l, this would yield a rate of ethanol oxidation of approximately 0.2 mg per h per kg of body weight. This should be compared with a total metabolism of approximately 100 mg per h per kg of body weight¹⁶. Erythrocytes therefore do not seem to play any significant role in the total metabolism of ethanol in man.

K. W. SMALLDON

Home Office Central Research Establishment,
Aldermaston, Reading, Berkshire

Received July 13, 1973.

- Brown, G. A., Neylan, D., Reynolds, W. J., and Smalldon, K. W., *Anal. chim. Acta*, **66**, 271 (1973).
- Smalldon, K. W., *Sixth International Meeting of Forensic Sciences, Edinburgh* (1972).
- Smalldon, K. W., and Brown, G. A., *Anal. chim. Acta*, **66**, 285 (1973).
- Jaffé, E. R., in *The Red Blood Cell* (edit. by Bishop, C., and Surgenor, D. M.), 397 (Academic Press, New York and London, 1964).
- Plueckhahn, V. D., *Med. J. Aust.*, **2**, 118 (1967).
- Plueckhahn, V. D., and Ballard, B., *Med. J. Aust.*, **1**, 939 (1968).
- Krauland, W., Vidic, E., Freudenberg, K., Schmidt, B., and Lenk, V., *Dtsch. Z. ges gerichtl. Med.*, **50**, 34 (1960).
- Krauland, W., Vidic, E., and Freudenberg, K., *Dtsch. Z. ges gerichtl. Med.*, **52**, 76 (1961).
- Glendening, B. L., and Waugh, T. C., *J. forens. Sci.*, **10**, 192 (1965).
- Robinson, A. E., and Camps, F. E., *Med. Sci. and the Law*, **10**, 69 (1970).
- Curry, A. S., Walker, G. W., and Simpson, G. S., *Analyst*, **91**, 742 (1966).
- Raskin, N. H., and Sokoloff, L., *Science, N.Y.*, **162**, 131 (1968).
- Trémolières, J., and Carré, L., *C.r. hebdom. Séanc. Acad. Sci. Paris*, **251**, 2785 (1960).
- Orme-Johnson, W. H., and Ziegler, D. M., *Biochem. biophys. Res. Commun.*, **21**, 78 (1965).
- Lieber, C. S., and DeCarli, L. M., *Science, N.Y.*, **162**, 917 (1968).
- von Wartburg, J. P., and Papenberg, J., *Psychosomat. Med.*, **28**, 405 (1966).

Table 1 Ethanol Concentration Change for Blood Samples During 16 Weeks at 20° C*

Inhibitor used in blood sample		Change in ethanol concentration (mg%)
None		-16.0
Sodium azide	(0.5% w/v)	+0.7
Sodium bisulphite	(0.5% w/v)	-2.3
Sodium dithionite	(0.5% w/v)	-2.2
Sodium nitrite	(0.5% w/v)	-0.2

* S.d. for ethanol changes ± 2 mg%.

Ribonucleoprotein Particle in Epidermis Cells as the Receptor for Juvenile Hormone

JUVENILE hormone maintains the larval character of insects¹. In the imago it controls maturation of oocytes and yolk deposition², and low doses of the hormone and its analogues

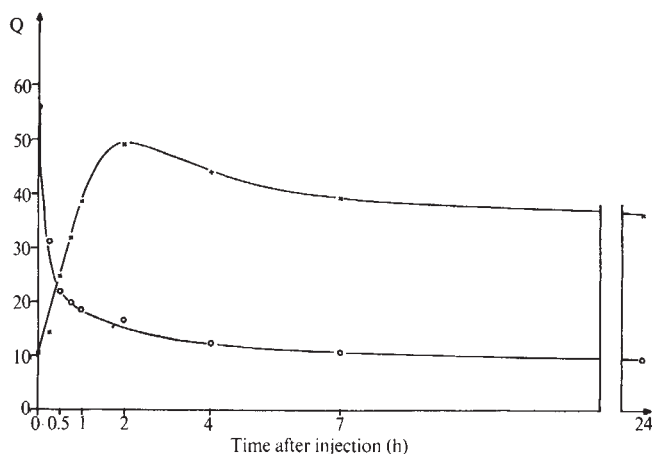


Fig. 1 Changes in distribution of H in the epidermis (x) and the remaining body parts (o) of pupae of *Tenebrio molitor* L with time. Pupae were injected with 10^{-8} mol l^{-1} labelled hormone in 1 μ l insect Ringer solution. After defined time periods the epidermis was separated from the remaining body and both body parts homogenized in 50 mM Tris (pH 7.4). After addition of 1 ml methanol the undegraded hormone was extracted with 3 ml petroleum ether. The products of degradation remained in the aqueous phase. After evaporation under nitrogen the lipophilic phase was separated by thin layer chromatography on silica and the band of the hormone eluted with dioxane. The radioactivity of the hormone band and the radioactivity of the aqueous phase were counted in a liquid scintillation counter. By relating with an external standard 100% yield was calculated. All operations were at 4° C. The ratio (Q) of undegraded hormone to total radioactivity in % is plotted against time.

cause persistence of the cutis of the pupae at the site of application³. Otherwise the insect develops to a normal imago. At higher doses there is an increase of hormone action which produces intermediates between pupa and imago⁴ and in this case too the main accent is on the cutis. We have tried to establish whether the epidermis cells which form the cutis are biochemical target organs for the hormone and its analogues. By definition a target organ accumulates the hormone against a concentration gradient and degrades it more slowly than non-target organs. We also searched for the molecular basis of this function.

As an analogue of the juvenile hormone we used [2,3-³H-propenyl]-10,11-epoxy-6,7-*trans*-2,3-*trans*-farnesylpropenyl-ether [designated as H]. The compound was labelled with ³H and had a specific activity of 28 Ci mmol⁻¹.

We compared the decomposition rate of H in the suspected target organ epidermis with those in the lymph and all remaining organs taken together. H is accumulated in the epidermis while at the same time it is decomposed quickly in the lymph and in the remaining organs (Fig. 1). This demonstrates that the epidermis cells of *Tenebrio molitor* pupae form the main target organ for the juvenile hormone.

Next we wanted to approach the molecular mechanism of H accumulation and reduction of H degradation rate in epidermis cells compared with the remaining organs. It was thought that H binds to a specific receptor in an analogous way to oestrogen receptor binding⁵. By tracing the radioactivity of bound and free H after separation on a 'Sephadex' column ('Sephadex' G200 fine), it is seen that H binds to a high molecular substance both *in vitro* and *in vivo* in homogenates of epidermis cells which were washed free of lymph. H can be recovered from this binding almost quantitatively and unaltered by extraction with lipophilic solvents. The H receptor complex has a molecular weight of $360,000 \pm 3\%$, as found by gel chromatography ('Sephadex' 6 B' and 'Sephadex' G200 fine) in Tris-buffer (pH 7.4), containing 0.4 M KCl, 10^{-3} M EDTA, 1 vol. % 'Triton X100'. Its isoelectric point is 4.4 as determined by the method of Radola⁶. Under the

conditions used (concentration of H and composition of buffer) only one single high molecular substance was found. Treatment with 6 M urea does not change the molecular weight of the complex. This indicates that the receptor is not composed of H-binding subunits. The specificity of the receptor is evident from its high affinity constant for H which is 4.4×10^{10} l mol⁻¹, calculated from the results of gel filtration using the method of Scatchard⁷. This explains the molecular basis for the accumulation of hormone analogue in the target organ (epidermis) from which the receptor was extracted.

In view of the low concentration of the receptor of 1.7×10^{-10} mol l⁻¹ in a pupa we have not attempted to isolate it for further structural studies. We tried to obtain information on its nature by digestion with specific enzymes. Assaying the hormone binding of the receptor by gel filtration it was seen that DNase and phospholipase C do not have any influence whereas trypsin and RNase destroys the receptor complex. No H-binding activity smaller than receptor could be detected. RNA and protein are constituents of the receptor.

The RNA content of the receptor suggests other functions as well as accumulation. This suggestion is supported by recently published results on oestrogen and androgen binding RNP particles of target organs of mammals⁸. These have a different molecular weight and therefore perhaps a different intracellular localization and function.

P. SCHMIALEK

Biochemie und Biophysik,
Zentralinstitut 5,
Universität Berlin

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¹ Wigglesworth, V. B., *Ql. J. microsc. Sci.*, **77**, 191 (1934).

² Wigglesworth, V. B., *Ql. J. microsc. Sci.*, **79**, 91 (1936).

³ Schmialek, P., Addendum, Wigglesworth, V. B., *Z. Naturforsch.*, **16**, b, 461 (1961).

⁴ Schmialek, P., *Insektenhormone—speziell das Juvenilhormon* (edit. by Asmis, H.) (Saladruk, Berlin, 1967).

⁵ Jensen, E. V., and DeSombre, E. R., *Annual Rev. Biochem.* (edit. by Snell, E. E.), **41**, 203 (Ann. Rev. Inc., Palo Alto, California, USA, 1972).

⁶ Radola, B. J., *Biochim. biophys. Acta*, **194**, 335 (1969).

⁷ Scatchard, G., *Ann. NY Acad. Sci.*, **51**, 660 (1949).

⁸ Liao, S., Liang, T., and Tymoczko, J. L., *Nature new Biol.*, **241**, 213 (1973).

Model for the Mating Protocol of Synchronously Flashing Fireflies

THE synchronous flashing behaviour of Asian fireflies has drawn the attention of Western naturalists for more than 100 yr, and was reported even earlier¹. Recent research and interest have been devoted to the physiological mechanisms of synchrony and the group display phenomenon, and have neglected consideration of individual fireflies within the swarms, their mating behaviour, and the significance of synchronizing to the individual. In this article I deal with these aspects of the biology of these fireflies. In Melanesia I observed eight species of *Pteroptyx*, the synchronizing fireflies, and fifteen species of *Luciola*, an allied genus. From these observations, published notes of others concerning *Pteroptyx* and observations of ninety-six species in other firefly genera, a model for the basic mating protocol of these confusing fireflies can be constructed.

Pteroptyx males and females gather in bushes, trees and hedges. Certain rhythmic flashes of males are emitted in synchrony with those of other males²⁻⁵. Other light emissions, not synchronized, include flickers and glows as males and females fly into and through foliage of the trees, participate in aerial chases, alight, and perch in close proximity⁶. There are more individuals in "swarm trees" than is superficially apparent; when shaken, the foliage sparkles with the glows and

flickers of those that had not been luminescing just previously. Copulating pairs are common in the trees and in the grass beneath^{3,6}.

The following model describes a generalized protocol that fits observations on several different species. For each of its six parts a general statement is made; supporting observations, arguments, and discussion then follow. Interspecific variation undoubtedly occurs in most or all steps, especially 4, 5 and 6. The basic elements of the model, listed approximately chronologically, are as follows. (1) Both sexes are attracted to the flash rhythm of males of their species. (2) Males in swarm trees detect approaching females and modify their luminescent behaviour in ways that stimulate females to land near them. (3) Recognition cues other than flash rhythm are detected as flying males and females near swarm trees. (4a) Females land near and observe specific males. (4b) Males land and flash in synchrony with nearby males. (5) Other behaviours bring individual males and females closer together. (6) A change of communicative channel occurs in the terminal stage of courtship before a male and female make physical contact.

(1) Males and females are attracted toward flashes with the appropriate species-specific rhythms and congregate in swarm trees that already harbour numbers of their species. They probably orientate to sites of highest light intensity that maintain the rhythm. Such sites will contain concentrations of sexually competent males and females; the likelihood of finding a mate there will be greater. (Basic components of this phase were discussed by J. B. and E. Buck⁴, based on their observations of Thailand *Pteroptyx*.) The formation of high densities in some trees while adjacent ones are vacant or nearly so is a common occurrence. Under certain circumstances males are "attracted to each other's light"⁴. Attraction of males to loci of higher intensity has been observed and experimentally demonstrated⁷. At lower densities fireflies are not randomly distributed over foliage but occur in clusters^{3,6}.

(2) Males in swarm trees detect the luminescence of approaching females and modify their behaviour to enhance the attractiveness of their own position in the tree. Females then may begin to select a landing site and a specific male or cluster of males. Case *et al.*⁷ reported that perched males aim their lanterns toward artificial or natural flashes "particularly if these sources are outside the aggregation". I found abrupt intensity changes in some recorded signals of a new species of *Pteroptyx* (sp. 22)⁶ that could be due to changes in lantern orientation (Fig. 1A). Females of many American species aim their lanterns at the flashes of their males⁸. Polunin's photograph⁹ illustrates the sharp angle at which *Pteroptyx* males hold their abdomens when flashing, and also shows that the head is extended and the eyes are maximally exposed. I have seen this extended head position in males of *Pteroptyx* spp., and in *Luciola obsoleta*, a species with aggregating behaviour similar to that of *Pteroptyx* spp.¹⁰. Perched *Pteroptyx* males (sp. 22) flicker in response to glows of females that fly over them. These females sometimes turn, swoop low over responding males and land within a few cm of them. Females of *Pteroptyx* (sp. 17) (probably *P. cribellata*) land near perched synchronizing males⁶.

(3) Cues other than the flash rhythm are probably detected as flying males and females near the swarm trees. There is considerable species-specific variation within the flashes that are synchronized (Fig. 1A–F). Some species, such as *Pteroptyx* (spp. 15 and 16) (Fig. 1C), have simple flashes. The emission of sp. 22 is a 12 Hz flicker lasting more than 1 s (Fig. 1A). That of sp. 19 (possibly *P. antennata*) is a four-modulation flicker with a rate of about 11 Hz and a duration of about 0.3 s (Fig. 1F). Three species, *Pteroptyx* spp. 17, 20, and 21⁶, have bimodal emissions (Fig. 1B, D, and E). The intensity relationships between the modes vary among the species but are fairly constant among males of each species. Interspecific discontinuity in modulation pattern and frequency is common in the signals of other fireflies and in the acoustic signals of insects^{8,11,12}, and is probably involved in reproductive isolation.

(4a) Females land near and (continue to) observe specific males. The flashing of a male is of prime importance in his selection as a mate by the female. If his luminescent performance did not enhance his own reproduction as he competes with conspecific males the genetic complex responsible for his behaviour would not be maintained. Mutation and selection in other contexts would erode it, and mass synchronous flashing would not persist, assuming that it had evolved in the first place.

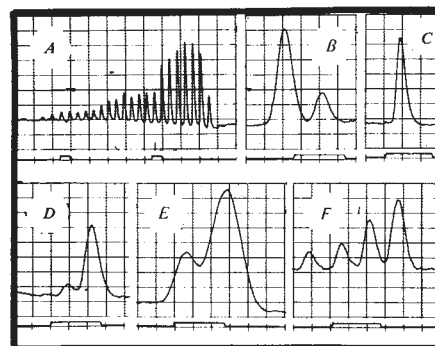


Fig. 1 Chart records of firefly flashes, recorded in the field. Each trace illustrates a single event; each is the unit that is synchronized and is presented rhythmically in continuous sequences. Vertical axis, relative intensity; horizontal axis, time, scale as indicated by marker below each trace. Marker duration is 0.12 s., and period (in A) is 1 s. A, *Pteroptyx* sp. 22. Arrow indicates abrupt and atypical intensity increment; in most recordings intensity increases gradually. B, *Pteroptyx* sp. 21; C, *Pteroptyx* sp. 16; D, *Pteroptyx* sp. 17 (*P. cribellata*?); E, *Pteroptyx* sp. 20; F, *Pteroptyx* sp. 19 (*P. antennata*?).

(4b) Males land and flash in synchrony with nearby males, and by synchronizing, increase the likelihood of obtaining females. It is necessary in the model that the benefit of synchronizing accrue to the individual male that is behaving in this manner (see argument in 4a). By synchronizing with his neighbours a perched male may enhance his probability of obtaining a female because he will not disrupt the species-specific rhythmic pattern and he contributes to enhancing the brightness of his locus on the tree. Both these factors may increase the likelihood of a female landing near him. In some species synchrony itself may have become an identification cue and females will not select males unless they are flashing in phase with their neighbours. Also, since a female's male progeny will tend to behave in the manner of their male parent (half their genes being derived from him), a female, on the average, will have a higher fitness if she combines her gametes with a male that demonstrates adequate performances as a male. If synchronizing with a neighbour enhances a male's reproduction, and this seems a certainty, then demonstration of this ability could become a component of courtship.

By flashing synchronously with a male that is already communicating with a female, an approaching male may be able to interfere and displace the other male. Such "interloping" is possible because the female is informed of the position of the male with whom she is communicating only during his flash; an approaching male that flashes in phase with him, and presents his own flash in a similar spatial position, may be able to complete the courtship. Interloping may be common in firefly mating. In experiments by Mast¹³ and Buck¹⁴ on *Photinus pyralis*, males orientated to and approached female flashes elicited by the flashes of other males. In Buck's experiments the males flashed in synchrony. I have made similar observations on *Photuris lineaticollis*, and captive males of *Photinus greeni* flashed in synchrony with an artificial light that was stimulating female flash responses¹⁵.

(5) Other behaviours bring individual males and females

close together after they have entered the swarm. Males of *Pteroptyx* spp. 17, 22, and perhaps 19, pursue flying glowing females, dart towards them and land near them. Females of sp. 22 fly closer to flashing males within a cluster after they have perched among them. Females of spp. 17, 19, and 22 begin luminescing during or following the emissions of nearby perched or flying males and attract them to their vicinity. Perched sp. 22 males flicker in response to glows of females that fly over them. These females sometimes turn, swoop low over responding males and land within a few cm of them (this behaviour was also placed under (2) above, though it may belong only here—the history of females behaving in this manner is unknown). Experimentally, I was able to attract males of four *Pteroptyx* species toward a penlight when I flashed it immediately after their flashes, both males flying closely about swarm trees and flashing in phase with the males in the tree, and males flying and flashing independently⁶. Some males approached to within a few cm of the light.

(6) A change of communicative channel may occur in the terminal stage of courtship. An interaction that is of singular importance for identifying courtship (for the observer) of species of other genera, in which mating behaviour has been studied, is the presence of a luminescent dialogue or of continuing luminescence up to the moment of physical contact¹⁶. This is not apparent and perhaps does not occur in most *Pteroptyx* species, but having spent many hours within swarms watching individuals I once saw a male (*Pteroptyx* sp. 19) receive a luminescent response to his signals from a female 30 cm away, walk to her and mount her immediately. In interactions mentioned above (see part (5)) males and females were brought to within a few cm of each other, but did not quickly draw closer. Also, males and females of spp. 17 and 22 were frequently found perched on the same leaf a few cm apart luminescing. Two examples were as follows. (a) During a flash dialogue a sp. 17 male landed 15 cm from the female. They luminesced for 4 min while he walked to within 4 cm of her, and no more was seen. (b) A signalling sp. 22 male landed on a leaf near a luminescing female. He walked about briefly, flashing; she continued to luminesce. All luminescence then stopped; 2 min later when I shone a light on them they were copulating. The conclusion from the observations and examples above is that if guided only by the insects' luminescence the observer does not see actual pair formation.

At some point when a male and female are very close, communication may be accomplished by non-visual signals. This could explain why so many non-luminescing individuals are perched in swarm trees. The intense competition and courtship disruption that can be expected to occur in dense *Pteroptyx* congregations could have favoured a change of communicative channel. Periodical cicada (*Magicicada* spp.), species that also form dense congregations and synchronize their signals, change from acoustical to visual signals during mating interactions^{17,18}. *Pteroptyx* spp. may switch to chemical signals; some fireflies are known to use pheromones¹⁹.

In the model described here individual males increase the likelihood of their own mating and the survival of the genes responsible for their behaviour, by their behaviour, flashing and otherwise. Previous explanations of synchronized firefly flashing have neglected to focus on the individual as the selected entity, proposed the existence of "indiscriminate centralized mating instead of the system of individual courtships"⁴, altruism for the benefit of individuals other than close kin⁷, or require genetic self sacrifice²⁰. There appears to be no justification for applying such explanation to firefly flashing behaviour. In the unlikely event that *Pteroptyx* fireflies are an exception to natural selection theory, as formulated and developed over the past century, this fact can best be determined by testing the hypotheses that selection theory suggests.

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J. E. LLOYD

Department of Entomology and Nematology,
University of Florida, Gainesville, Florida 32605

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- ¹ Buck, J. B., *Q. Rev. Biol.*, **13**, 301 (1938).
- ² Haneda, Y., in *Luminescence of Biological Systems* (edit. by Johnson, F. H.), 360 (Am. Assoc. Adv. Science, Washington, 1955).
- ³ Haneda, Y., *Sci. Rep. Yokosuka City Mus.*, **12**, 4 (1966).
- ⁴ Buck, J. B., and Buck, E., *Nature*, **211**, 562 (1966).
- ⁵ Buck, J. B., and Buck, E., *Science, N.Y.*, **159**, 1319 (1968).
- ⁶ Lloyd, J. E., *Environ. Entomol.*, **2** (in the press).
- ⁷ Case, J. F., Hanson, F. H., Polunin, I., and Barnes, A. T., *Am. Zool.*, **12**, 683 (1972).
- ⁸ Lloyd, J. E., *Univ. Mich. Misc. Publ.*, **130**, 1 (1966).
- ⁹ Zahl, P. A., *Nat. geog. Mag.*, **140**, 45 (1971).
- ¹⁰ Lloyd, J. E., *Coleop. Bull.*, **26**, 155 (1972).
- ¹¹ Alexander, R. D., *Evolution*, **16**, 443 (1962).
- ¹² Spooner, J. D., *Anim. Behav.*, **16**, 197 (1968).
- ¹³ Mast, S. O., *J. Anim. Behav.*, **2**, 266 (1912).
- ¹⁴ Buck, J. B., *Science, N.Y.*, **81**, 340 (1935).
- ¹⁵ Lloyd, J. E., *Coleop. Bull.*, **23**, 35 (1969).
- ¹⁶ Lloyd, J. E., *A. Rev. Entomol.*, **16**, 97 (1971).
- ¹⁷ Alexander, R. D., in *Animal Communication* (edit. by Sebeok, T. A.), 172 (Indiana University, Bloomington, 1968).
- ¹⁸ Alexander, R. D., and Moore, T. E., *Univ. Mich. Misc. Publ.*, **121**, 29 (1962).
- ¹⁹ Lloyd, J. E., *Environ. Entomol.*, **1**, 265 (1972).
- ²⁰ Wynne-Edwards, V. C., *Animal Dispersion in Relation to Social Behaviour*, 198 (Hafner, New York, 1962).

Carrot Embryogenesis from Frozen Cultured Cells

MANY plant tissue cultures change in growth rate, chromosome cytology and morphogenic potential during repeated subculture¹⁻³. Controlled freezing and low temperature storage of cultured plant cells might enable the characters of newly initiated cultures to be preserved. Cell preservation at the temperature of liquid N₂ (-196° C) has been successful with animal cells⁴ and preliminary work with plant cells has shown the promise of this approach⁵⁻⁷. Latta has reported regrowth of cultures of carrot (*Daucus carota* L.) following freezing for

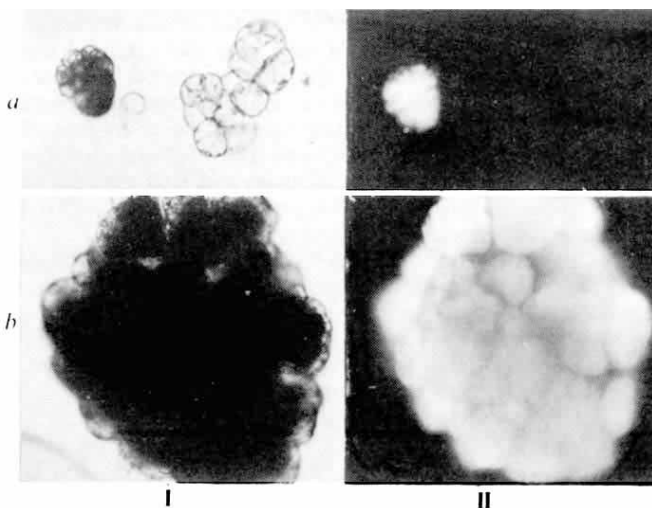


Fig. 1 Twin pictures taken with tungsten light (I) and ultra-violet lighting (II) after staining with fluorescein diacetate. Cells preserved at -196° C for 35 d before thawing. a, Portion of culture containing a small dense aggregate and a loose aggregate of large cells. Dense aggregate of small cells survives, large cells do not survive. b, An aggregate initiating embryos which shows complete survival.

up to 2 months at -40°C or at -196°C but did not assess the percentage of cells surviving⁶.

Here we have examined various aspects of the freezing-thawing procedure using high embryogenic cell lines of domestic and wild carrot. We have assessed percentage survival by the use of fluorescein diacetate⁸, demonstrated the retention of a high level of embryogenic potential by the retrieved cells and obtained from them young carrot plants showing normal morphology and development.

Actively-growing (5 to 15 d after subculture) suspension cultures (50 ml cultures in 250 ml wide-mouthed Erlenmeyer flasks) in Murashige and Skoog's (MS) medium containing 0.5 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D)³ were allowed

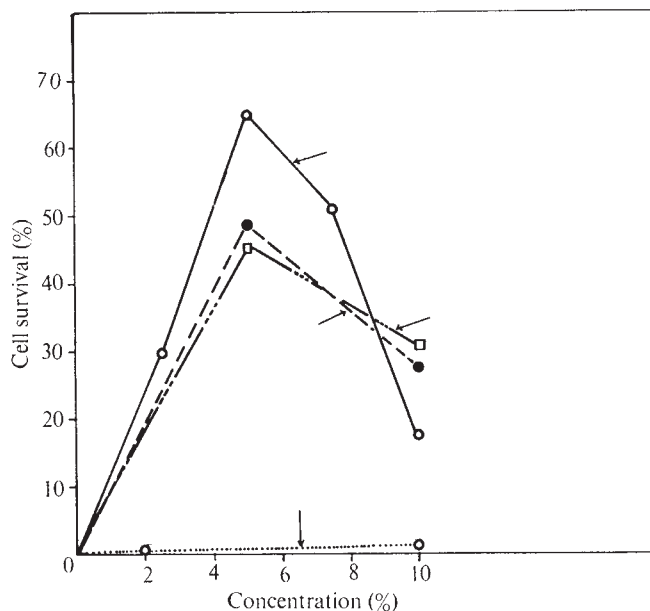


Fig. 3 Cell survival in the presence of different protective agents. Cooling rate $1.8^{\circ}\text{C min}^{-1}$, storage -196°C for 7 d, initial thawing rate $120^{\circ}\text{C min}^{-1}$. $\circ$ — $\circ$, DMSO; $\bullet$ — $\bullet$, DMSO + glycerol; $\square$ — $\square$, glycerol; $\circ$... $\circ$, sucrose.

to settle in an ice-bath for 20 min and concentrated by aseptic removal of 20 ml of supernatant medium. In aseptic conditions, aliquots of 0.8 ml suspension culture were transferred to sterile plastic ampoules ($1.2 \times 4.5\text{ cm}$) followed by 0.8 ml sterile medium containing protective agents at twice the chosen concentration (added at 0 to 1°C in four portions of 0.2 ml in 20 to 60 min). Several protective agents were tested at the following final concentrations: dimethylsulphoxide (DMSO), 2.5, 5, 7.5 and 10%; glycerol, 5 and 10%; DMSO + glycerol, 5 and 10% of each; and sucrose, 10%. After the addition of protective agent the ampoules were kept in the ice-bath for 15 min to 3 h and then frozen at various cooling rates (0.8 , 0.9 , 1.3 , 1.8 , 2.3 , 3.0 and $4.0^{\circ}\text{C min}^{-1}$) in a Union Carbide BF-6 Biological Freezer. Ampoules were removed from the freezer when they reached -70°C and subsequently stored at -20° , -78° or -196°C . In each experiment each treatment was represented by at least nine ampoules, and each experiment has been successfully repeated.

The ampoules were thawed to the liquid state at the following initial thawing rates: $120^{\circ}\text{C min}^{-1}$ (ampoules in a water bath at 37°C); 12 – $14^{\circ}\text{C min}^{-1}$ (in air at room temperature); 1.5 – $2.0^{\circ}\text{C min}^{-1}$ (immersed in alcohol chilled to -78°C and allowed to warm at room temperature); and $0.5^{\circ}\text{C min}^{-1}$ (dry ice subliming at room temperature). The thawed ampoules were transferred to an ice-bath, excess medium removed after centrifuging at 0°C and the cells washed three times with 1.2 ml fresh sterile medium chilled to 0°C acting for 1 min between centrifugings.

The cell suspension cultures consist of dense aggregates of small cytoplasm-rich cells and larger more highly vacuolated cells present as single free cells or in few celled aggregates. These large cells either do not divide further or divide with low frequency and growth of the culture takes place predominantly by cell division in the more superficial cells of the dense aggregates³. It is these dense aggregates which give rise to embryos as the culture ages and particularly on subculture to an auxin-free medium.

Cell survival in the suspension after thawing was assessed by mixing one drop of the washed cell suspension with one drop of 0.01% fluorescein diacetate on a microscope slide⁸. Cell counts were made under the microscope in ten fields in each of two slides per ampoule, first in tungsten light (total count) and then in ultraviolet light. Cells showing fluorescence were counted as surviving and expressed as a percentage of the total

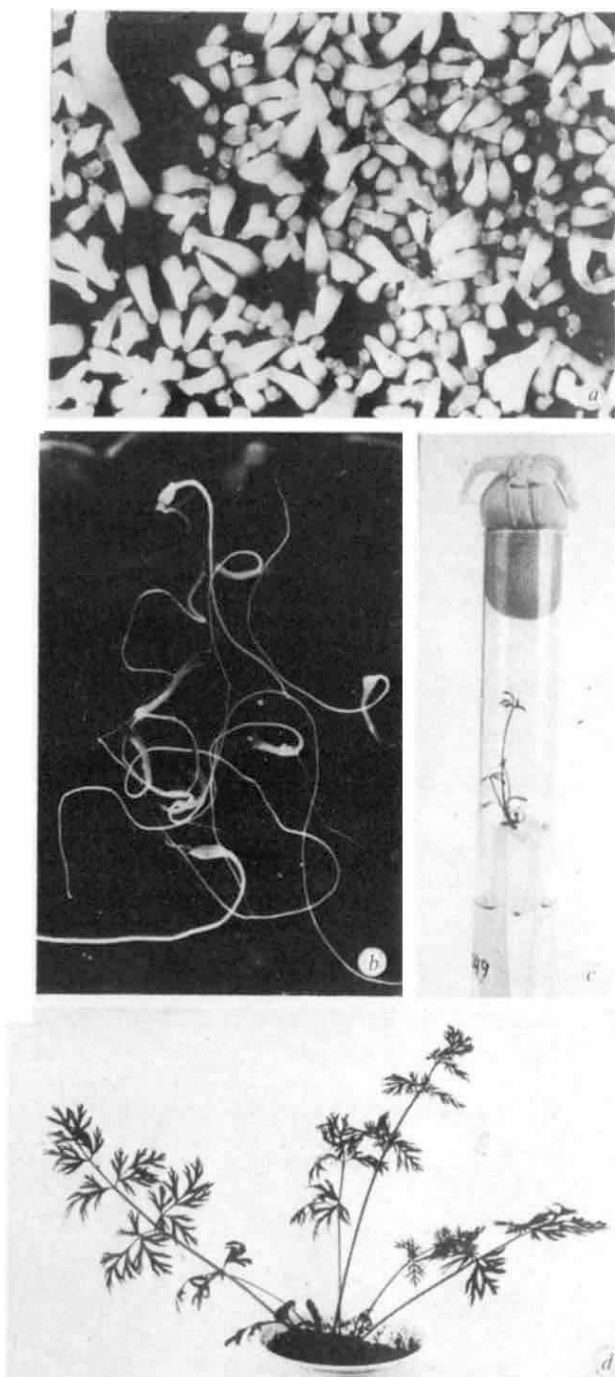


Fig. 2 *a*, Embryos developing in Halperin's medium in a culture retrieved after 45 d at -196°C . *b*, Seedlings ready for transfer to filter paper bridges in an older culture such as *a*. *c*, Seedling from *b* developing on filter paper bridge. *d*, Carrot plant developed from a culture retrieved after 3 d storage at -196°C .

count. Cell numbers in the dense aggregates were estimated by up and down focusing and are, therefore, only approximate. The values for cell survival, however, are probably underestimates of survival by the cells of the dense aggregates as the larger released vacuolated cells of the cultures always showed poor survival (Fig. 1a). In the most favourable conditions established, there was often complete survival of all cells of individual aggregates. In some instances aggregates had begun to initiate embryos and the cells of these young embryos showed a high level of survival (Fig. 1b).

The bulk of the thawed suspension in the ampoule was transferred to a 100 ml wide-mouthed Erlenmeyer flask containing 10 or 25 ml of the MS medium containing 2,4-D and incubated on a horizontal shaker at 25° C. Whenever the cell survival was at or above 35%, culture was achieved and the retrieved culture was capable of a similar maximum growth rate and achieved a similar maximum cell density to the parent culture. But irrespective of survival percentage or inoculum density the first passage culture following thawing showed a long lag phase (20 to 30 d) before cell division began. Retrieved cultures were tested for embryogenic potential by subculture to Halperin's medium⁹ (without added 2,4-D) and by days 6 to 7 of the second passage in this medium produced large numbers of heart-shaped and torpedo-shaped embryos (Fig. 2a). After a further 15 to 25 d of culture these had given rise to seedlings with well developed cotyledons and roots (Fig. 2b); and such seedlings were successfully transferred to filter paper bridges (Fig. 2c) in tubes containing half-strength Halperin's medium and after an additional 20 d could be transplanted to pots of seedling compost where they developed into healthy plants of normal morphology (Fig. 2d). There was no evidence of impaired embryogenic potential in the retrieved cultures.

The study of the influence of freezing and thawing regime and of protective agents was carried out on wild carrot with culture lines having chromosome numbers of 18 or 35, and on cultivated carrot with two culture lines having chromosome numbers of 17 and 18. Plants have been raised from each line in retrieved cultures.

Examination of large numbers of metaphase plates in squash preparations has yielded no evidence of any effect of freezing preservation on the karyotype.

Using a cooling rate of 1.8° C min⁻¹, preservation at -196° C for 7 d and an initial thawing rate of 120° C min⁻¹, it was shown that survival was less than 0.1% in absence of added protective agent (except the 2% sucrose in the medium), was little improved

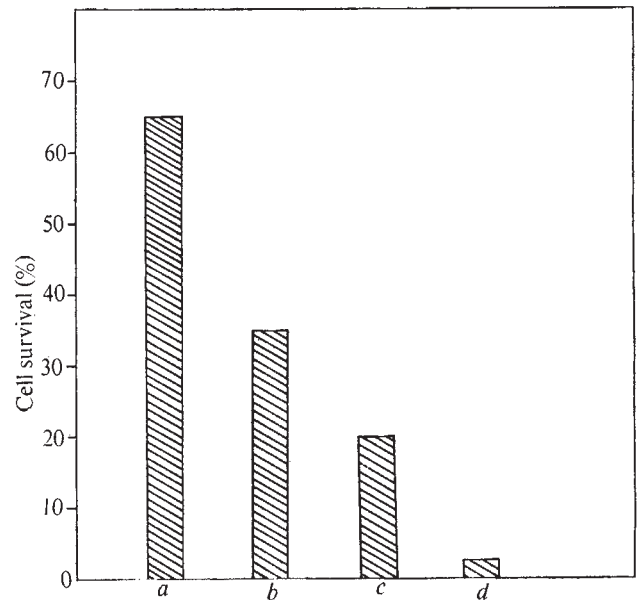


Fig. 5 Effect of thawing procedure on cell survival. Techniques of obtaining different initial thawing rates are given in the text. Protective agent 5% DMSO, cooling rate 1.8° C min⁻¹, storage 7 d at -196° C. a, 120° C min⁻¹; b, 12 to 14° C min⁻¹; c, 1.5 to 2.0° C min⁻¹; d, 0.5° C min⁻¹.

by addition of higher levels of sucrose, but was greatly increased by appropriate concentrations of DMSO and glycerol (Fig. 3). Further study is needed to determine whether different cooling rates are required for maximum survival with different levels of protective agents, and whether there is any interaction between cooling and warming rates.

Using 5% DMSO as protective agent and 120° C min⁻¹ as thawing rate, it was shown that highest survival was obtained at a cooling rate of 1.8° C min⁻¹ (65 to 68% cell survival; Fig. 4). Cell survival was best at the highest thawing rate (immersion in water bath at 37° C) tested (Fig. 5).

Cells can be stored at -78° C but there is progressive decline in cell survival as storage is prolonged. No decline in cell survival has been recorded during storage at -196° C for over 100 d. Storage at -20° C is very ineffective.

Although a high culture survival rate has been achieved, there is reason to hope that this can be still further enhanced. It is also necessary to test the reliability of the present index of cell survival by studying the plating efficiency at low density¹⁰ of the retrieved cells. The fact that morphogenic potential is unimpaired by the freezing preservation technique developed suggests that it may be possible to establish cell and tissue banks of particular plant genotypes by this approach.

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K. K. NAG
H. E. STREET

Botanical Laboratories, University of Leicester,
Leicester LE1 7RH

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- ¹ Torrey, J. G., *Physiologia Pl.*, **20**, 265 (1967).
- ² Nag, K. K., and Johri, B. M., *Phytomorphology*, **19**, 405 (1969).
- ³ Smith, S. M., and Street, H. E., *Ann. Bot.* (in the press).
- ⁴ Whittingham, D. G., Leibo, S. P., and Mazur, P., *Science, N.Y.*, **178**, 411 (1972).
- ⁵ Quatrano, R. S., *Pl. Physiol., Lancaster*, **43**, 2057 (1968).
- ⁶ Latta, R., *Can. J. Bot.*, **49**, 1253 (1971).
- ⁷ Bannier, L. J., and Steponkus, P. L., *Hort. Sci.*, **7**, 194 (1972).
- ⁸ Widholm, J. M., *Stain Tech.*, **47**, 189 (1972).
- ⁹ Halperin, W., *Am. J. Bot.*, **53**, 443 (1966).
- ¹⁰ Street, H. E., in *Plant Tissue and Cell Culture* (edit. by Street, H. E.), 191 (Blackwell, Oxford, 1973).

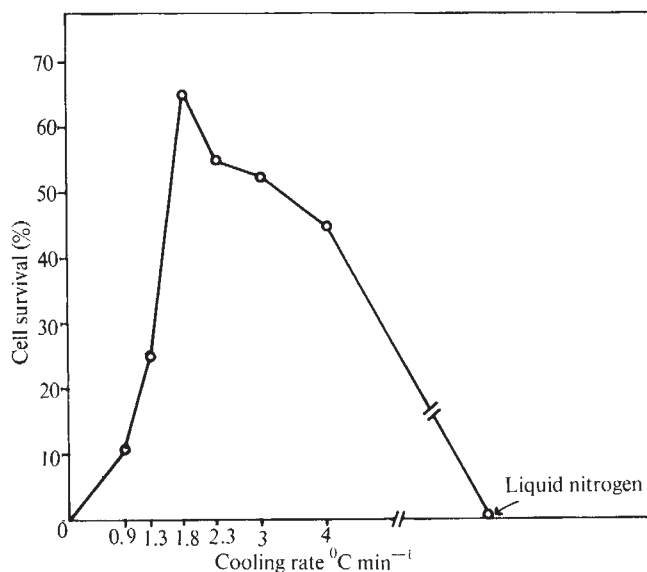


Fig. 4 Cell survival at different cooling rates. Protective agent 5% DMSO, storage -196° C for 7 d, initial thawing rate 120° C min⁻¹. Liquid nitrogen (nil survival) represents direct immersion of ampoules in the liquid nitrogen.

BOOK REVIEWS

Pacific Arts of Navigation

We, the Navigators: The Ancient Art of Landfinding in the Pacific. By David Lewis. Pp. xviii+346 (13 plates). (Australian National University: Canberra; Angus and Robertson: United Kingdom, Europe, Middle East and Africa, January 1973.) £5.

ALMOST from the moment of their discovery by Europeans the islands of the South Pacific have been the object of unflagging fascination. The idea of an earthly paradise, free of the constraints and artificialities of so-called civilization, has exerted a powerful attraction, while at the same time the European colonizers crushed with their philistinism what drew them there. Of course, a good deal of the culture and traditions of these islands has been painstakingly recorded by armies of anthropologists even as it was demolished. Surprisingly though, the aspect of life which seemed perhaps the most central to the culture of Oceania, seafaring and navigation, has been relatively sparsely described. In this book David Lewis, doctor and small-boat sailor from New Zealand, writes about his studies of indigenous inter-island navigation, taking one of the last opportunities to do so before the practices and traditions are finally forgotten.

Dr Lewis set himself two main tasks, to record the ancient arts of the Pacific navigators, and then to consider again in the light of his understanding the problems of how the islands first came to be settled. When Captain Cook took Tupaia aboard his ship in Tahiti he was singularly fortunate to have acquired the cooperation of an island chief and navigator-priest. Cook and the company of the Endeavour seemed mildly surprised by the extent of Tupaia's geographical knowledge, extending from Fiji in the west to the Marquesas in the east, a span roughly the size of the Atlantic. They seemed quite interested when Tupaia at any stage of the subsequent voyage could point to the position of Tahiti. However, no one seemed to think of asking him any details of how he did this or about many other aspects of his navigational methods. For some reason the idea that illiterate stone-age people without charts or instruments of any kind could have developed an effective and intricate art of navigation

simply did not occur to the eighteenth and nineteenth century European mind, even though it was clear that long voyages took place between islands which were mere specks in the sea, hundreds of miles apart. In fact it is probable that for more than a thousand years before the great age of European world exploration both boat building and navigational techniques in Oceania were substantially more advanced than they were in Europe.

What has emerged into a few Western minds rather recently is just how sophisticated and effective the ancient navigational methods of Oceania were. The technique consists mostly of dead reckoning and is based on a sidereal compass. Thus, direction is defined principally by rising and setting points of stars, which are used in actuality at night, but also conceptually by day. The star courses between many pairs of islands are part of the material to be learned in the extensive apprenticeship of a navigator. But though the sidereal compass is important, the core of navigational technique seems to be a very highly trained spatial understanding so that on a voyage the navigator continually updates his understanding of where he is in relation to the islands he knows, typically choosing one particular reference island, off to one side of the course, which he will not actually sight on that particular voyage. By maintaining his course and continually thinking of the direction of the reference island he can mentally follow the progress of the voyage. There is a great deal more to the method than this: elaborate understanding of currents, judgment of speed, maintenance of direction relative to wind and waves, subtle discriminations of sea colour over reefs and cloud patterns over islands, refraction and reflexion patterns of waves, knowledge of bird behaviour and many other factors are all combined in the expertise of the navigator. Lewis has recorded comprehensively and carefully as many of these traditions as possible from widely spaced sources in Oceania, and taken voyages with some of the few remaining navigators from the Caroline Islands. Some of the material is rather similar to that contained in another excellent recent book

on the subject, *East is a Big Bird*, by Thomas Gladwin. (The Big Bird is the name for Altair, the star which rises almost due east of the Caroline Islands.) But Lewis's book is wider ranging, more systematic and with more of a feeling for what it is like to practise these navigational arts; something which is no doubt helped by the fact that the author himself is an experienced and intrepid sailor.

The acceptable limits of accuracy of landfalls can be as fine as 7.5 degrees over open sea and across variable currents, and the fact that there are still a few navigators capable of making such voyages of 500 miles or more testifies to the efficacy of these traditions. As Lewis points out, since the Polynesians and Micronesians were clearly armed with powerful methods of navigation, and could provision seaworthy craft for several weeks, and had highly developed skills of seamanship, the question of how the Pacific Islands were settled ceases to be much of a mystery. A crucial factor encouraging deliberate exploration must be the confidence of being able to return, and Lewis makes it fairly clear that this would have been possible, even over the longest necessary ranges of two thousand miles, for example, from Tahiti to Hawaii. There seems to be agreement that the main routes of island settlement were from South East Asia via Indonesia, and the archaeological evidence seems to indicate that even the more distant parts of the Pacific were settled nearly fifteen hundred years ago. But an understanding of indigenous navigational skills solves the problem of how coastal fishermen could have drifted helplessly to populate so many scattered islands. It is now possible to contemplate elaborate seafaring skills as the basis of exploration, of continued inter-island communication, and of a stable, intricate and evidently satisfying way of life. This book, and indeed the whole topic of Pacific navigation, is of consuming interest to anyone concerned with anthropology, with the psychology of spatial conceptualization, with seafaring, or indeed with the history of man's struggle to find appropriate ways to live. Lewis's book is first rate and it is profoundly regrettable that the traditions which he

and a few others have tried to record in writing are already almost extinct.

KEITH OATLEY

On Rattlesnakes and Men

Rattlesnakes: Their Habits, Life Histories and Influence on Mankind. By Laurence M. Klauber. Volume 1: Pp. xxx+1-740. Volume 2: Pp. 741-1533. (University of California: Berkeley, Los Angeles and London, February 1973.) £22.50 two volumes.

LAURENCE KLAUBER was a professional engineer whose hobby was herpetology. He was unusual as an amateur who not merely made a significant contribution to knowledge but who also influenced the methods of study of the professionals in the field. In these volumes we find many examples of Klauber's application of statistical methods to the study of variation and ontogenetic change and the discrimination and description of species and subspecies.

One of my strongest impressions on reading this work is that it displays the endearing enthusiasm of a critical but kindly man. Klauber obviously enjoyed collecting all kinds of information about rattlesnakes, both factual and fictional. He was neither hurrying to produce an impressive pile of publications nor striving to build himself a reputation; he took his time studying the animals in the field and laboratory, talking and corresponding with many people, and searching through newspapers, magazines, histories, travel stories, ethnographic and medical literature as well as the technical herpetological literature. He avoids holding correspondents up to ridicule by omitting their names where they give him highly doubtful information but attributes absurd printed statements "since the author has placed himself on record".

Rattlesnakes range from parts of southern Canada through the New World tropics to the northern provinces of Argentina. Klauber recognizes thirty-one species and seventy subspecies. The work is addressed to non-technical readers, but sufficient technical taxonomic information is given to enable the reader to make a detailed identification of any rattlesnake. There are keys, maps and ecological information for all forms and nearly all are illustrated with good photographs.

Few quantitative aspects of rattlesnakes have not been counted, measured, weighed or timed. Variation within and between populations is studied as well as in relation to ontogeny and ecology; characters are examined pair by pair for correlations. Amongst other interesting investigations we find an account of relative fang sizes of different species, fang replacement and changes of shape and proportionate size during growth. The development of

the rattle, the feature which gives these snakes their name in all languages, is described in detail. Klauber did these statistics in the days of mechanical calculating machines. His tens of thousands of raw data items must be waiting for someone with an electronic computer to extract yet more information from them.

The habits of the different species are considered at some length, both as they relate to the modes of life of the snakes and as they bear upon the risks of snakebite. There are sections on population density, control measures, collection, shipment and rearing and care of rattlesnakes. The venoms, their effects and treatment naturally receive generous attention and there is a discussion of the difficulties of compiling reliable snakebite statistics. If Klauber has no first hand knowledge of some aspect he reviews other people's work or, at the very least, mentions it and gives references.

This is a book about people as well as rattlesnakes. In addition to special chapters on folklore and Amerindian beliefs the book is sprinkled with examples of such human failings as ignorance, gullibility, foolhardiness and guile. The history of our knowledge is traced and many early anecdotes are recounted.

Such a compilation about a single group of animals, with more than 4,000 references, must make the group attractive to other researchers with evolutionary interests in various fields. Reading the text suggests many matters which would merit further study. The volumes are well produced, I found barely half a dozen typographical errors in 1,500 pages. They stand as a splendid memorial to a man who aroused the animosity of none and the respect and love of many.

GARTH UNDERWOOD

Facts of Malnutrition

The Hungry Planet: The Modern World at the Edge of Famine. By George Borgstrom, Pp. xviii+552. (Collier: New York; Collier-Macmillan: London, March 1973.) £1.25.

"ONE and a half billion people are undernourished, and the diets of an additional one billion are deficient in one or several key nutrients" (page ix).

"Recent studies have established the prevalence of hunger and malnutrition among ten or even more millions in the United States" (page 55).

"Ecuador, Colombia, Bolivia and Guatemala [with protein consumption of 50-57 g/person d⁻¹] get hardly more than half the protein they need" (page 288).

"More deserts have been created by man than ever new land was gained through irrigation" (page 510).

It is difficult to tell whether the author really intended this to be regarded as a scientific book. He makes these and many other extraordinary statements without advancing any evidence for them, except for confused reading lists, consisting mostly of popular books, not scientific journals.

The legend that half the world is malnourished (the author throws in an extra billion people for good measure), which originated with the Food and Agriculture Organization (FAO), was later shown to rest only on the evidence that half the world did not, indeed, eat as much as the inhabitants of Western Europe (many of whom are dying from diseases brought on by overeating).

Recently, however, FAO has had a change of heart. Dr Pawley, Director of FAO Policy Advisory Bureau, has indicated that during the coming century we could without serious difficulty raise world food production to fifty times what it is now¹.

The author quotes 3,000 calories d⁻¹ as the minimum requirement for a man of 70 kg (too low), makes some adjustment for Indian body weights, but then hopelessly confuses requirements per adult man and requirements per head of the population, ending up by estimating Indian minimum requirements at 2,400-2,500 (the correct figure is about 1,650). Food supplies now available in India would give an average of 1,900, which if equally distributed would suffice to provide this minimum, with a small margin to spare.

I have published an estimate that the proportion of the population of India receiving less than minimum calorie requirements is about 25%². The distribution of income to landless labourers, poor tenant farmers, and the lower castes generally (something which only the rulers of India can correct) leaves 25% hungry.

The author produces no evidence on protein requirements; but his statements on Latin America imply a requirement of about 100 g/person d⁻¹.

Few serious estimates go higher than Dr Gopalan's³ 38 g (of protein as consumed, with conversion factor to reference protein averaging 0.65); and some estimates would only be two-thirds of this figure. Dr Gopalan, and several others, have recently brought about an important change of opinion on the question of protein requirements. While clinical manifestations of protein deficiency, in many countries, are all too clear, these are rarely due to a lack of adequate protein in the diet. When the diet is deficient in calories, the patient cannot assimilate the protein. What the developing countries need is more of their traditional diets, with only very limited protein supplementation. The need for animal protein, as such, is minimal.

The author makes no reference to the fact that food production in the developing countries is increasing substantially faster than their population. The food situation in Russia, China and Cuba he finds satisfactory. He sympathizes with the Japanese for the overcrowding and traffic congestion in their cities; it has not occurred to him that these could be cured by decentralization.

Subject to the above substantial qualifications, this is an interesting book, containing much information on food, agriculture, geography, hydrology and so on.

COLIN CLARK

¹ *Ceres*, July–August 1971.

² *Economic and Political Weekly*, Bombay, September 30, 1972.

³ Gopalan, *Am. J. clin. Nutr.*, January 1970.

Topics in Zoology

Evolutionary Biology. Edited by Th. Dobzhansky, M. K. Hecht and Wm C. Steere. Vol. 6. Pp. xiii+445. (Appleton Century Crofts: New York, October 1972.) \$19.95.

FEW workers can have provided basic contributions to the guiding principles and knowledge of as many branches of zoology as has Dr George Simpson. This volume is dedicated to him and contains papers from eighteen workers in the fields in which he carried out his own researches—evolution, zoogeography, palaeontology and taxonomy.

Williams contributes an extremely penetrating and thought-provoking study of the zoogeography of the *Anolis* lizards of the West Indies. He first suggests three “rules” based on the work of Schoener, which predict the sizes of *Anolis* species. He then shows that these results can be used to reconstruct the sequence of arrival of the eleven species in Puerto Rico, and that this sequence is confirmed by studies of their phyletic relationships.

In an enjoyable style, Gould discusses the controversy as to whether the Lower Liassic *Gryphaea* species show a trend to increased tightness of coiling. He notes that in fact there is no evidence that any individuals were unable to open their shell, and his analysis of the data shows that there was no increase in coiling but merely an increase in size.

Three papers consider the relationships between palaeontological data, continental drift and palaeogeography. As Colbert shows, the discovery of the hippo-like reptile *Lystrosaurus* in Antarctica is strong evidence for a Triassic Pangaea, while McKenna demonstrates that comparisons of the mammal faunas of North America, Europe and Asia in the early Tertiary make simple sense when added to recent studies of the history of the connexions of these areas. Finally, Hoffstetter suggests that the easiest solution to the

problem of the relationship between the South American and African rodents and monkeys is direct early Tertiary migration to South America across a then-narrower South Atlantic.

The evolutionary and phyletic significance of character complexes is discussed in several papers. McDowell suggests a series of adaptive stages in the evolution of tongue structure in lizards, and their significance in the problem of the origin of snakes. MacIntyre discusses the morphology of the petrosal of Late Cretaceous mammals and recognizes in them a primitive pattern which he terms “trisulcate”. Romer gives an integrated account of his view that the special myological, embryological and neurological aspects of the pharyngeal region of vertebrates are relics of its importance in filter-feeding in early vertebrate history.

Several authors discuss aspects of human evolution. For example, Washburn compares the indication of different types of data on human evolution, while Neal and Schull note how little is known on the important subject of the different fertility of different races and populations of man. Lewontin presents the first estimate of the pattern of human variation, and obtains the remarkable result that only 6.3% is inter-racial, and more than 85% is not merely inter-racial but is contained within populations.

The levels of significance and interest of the papers in the volume seem to be well above average, and reflect the respect and affection with which Simpson is regarded by his fellows.

BARRY COX

Calibration Statistics

Uncertainty, Calibration and Probability: The Statistics of Scientific and Industrial Measurements. By C. F. Dietrich. Pp. xii+411. (Adam Hilger: London, May 1973.) £18.

THERE is no doubt of the need for a book on the statistical aspects of calibration. This privately published venture is, however, unlikely to prove to be that book. The greater part of the space is taken up with clearly drafted and beautifully printed derivations of basic statistical thinking which have been known for a long time and by now could be taken as definitive for use in a particular context. This aspect is supported by absence in the bibliography of references to the statistical journal literature of the last decade: for example, the work of Grubbs, Krutchkoff, Hahn and Thompson in the instrumentation and industrial field and Berkson in the biomedical field. For the quite different field of model calibration, for example, transportation projects and macro-econometrics, there is a separate literature but which is still part of the statistical approach to

calibration. However, the sub-title of this book may relieve the author of responsibility for these latter aspects. What is required is a treatment of the whole field of calibration problems so as to show the unified statistical approach to their understanding and resolution.

W. R. BUCKLAND

On Foetal Growth Rate

On Fetal Growth Rate: Its Variations and Their Consequences. By Margaret Ounsted and Christopher Ounsted. Pp. xii+204. (William Heinemann: London; J. B. Lippincott: Philadelphia, 1973.) £3.60.

THIS is a useful book. It contains careful thought and represents a personal dedication to the study of foetal development. Much of present knowledge is summarized, and the authors present results from their own detailed study.

The following are some of the topics covered. The social and biological factors related to birth weight; the construction of birth weight standards; the development of the placenta; the results of breeding experiments in other mammals; familial patterns of birth weight; perinatal mortality and morbidity related to birth weight; neonatal and postnatal development patterns. There is a chapter which discusses possible theoretical mechanisms determining foetal development and a final chapter pointing a way to practical developments and further research.

The book, however, has some weaknesses. To begin with, the title is unfortunate. What the authors actually discuss is weight at birth and not rate of weight growth. A further confusion is created by calling the standards of birth weight for length of gestation, “growth” standards. This implies that, for example, the (intrauterine) weight distribution at 40 weeks of gestation of those babies born at 43 weeks is identical with the birth weight distribution of babies born at 40 weeks—which is almost certainly not true.

There is also a problem resulting from the basic design of the authors’ own study. They chose three groups, large for dates (LFD), small for dates (SFD) and a random control group; groupings which are relatively inefficient at predicting the risks of perinatal mortality. For example, Rantakallio¹ shows that perinatal mortality rates vary with birth weight but relatively little with gestation. Yet in their final recommendations the authors advocate presentation of perinatal mortality rates by the LFD and SFD classifications—a clearly inefficient procedure. Both Rantakallio and Butler and Alberman² show that at 2,500 g the perinatal mortality is about twice the average. Thus the old “rule of thumb” which treated this birth weight as a

danger threshold was perhaps not so far wrong. It would be a pity if this book, which may well become a standard reference, were to condone and help to perpetuate imprecise and often counter-productive terminologies and classifications. HARVEY GOLDSTEIN

¹ Rantakallio, P., *Annls Paediat. Fenn.*, **14**, 66 (1968).

² Butler, N. R., and Alberman, E. D., *Prenatal Problems* (Livingstone, Edinburgh, 1969).

Organizing Books

Classified Library of Congress Subject Headings. Edited by James G. Williams, Martha L. Manheimer and Jay E. Dailey. Volume I: *Classified List*. Pp. v+250. Volume II: *Alphabetic List*. Pp. v+250. (Marcel Dekker: New York, November 1972.) \$25 each volume.

THE Library of Congress system, devised for the organization of knowledge in the collections of that library, consists of two parts: a classification scheme for the arrangement of books and a subject headings list for the arrangement of alphabetical catalogues. Nowadays we are aware that such a system should be integrated; that class numbers and subject headings are merely different ways of expressing the same concepts; that the cross references in an alphabetical subject catalogue should reflect the structure of a classification scheme. We are able to design an index language that can be used both for the shelf arrangement of documents and for the construction of any type of catalogue or index. When the LC system was devised the two parts were taken to have different aims and principles. They were developed independently and bear little relation to each other. Even as separate entities they are not particularly good examples of their kind. The classification scheme has never claimed to show the structure of knowledge as seen by specialists. Its out-of-date method of attempting to enumerate all the possible subjects of documents was doomed to failure from the start and inevitably leads to inconsistency and cross-classification. The subject headings notoriously fail to obey even the few rules they profess.

The aim of the work under review is to improve the efficiency of libraries using the LC system. It is addressed to the research worker as well as the librarian and is meant to be used by both. Volume I is a completely new contribution in that it arranges the subject headings in the order of the classification scheme. The preface admits that "research now confirms what reference librarians and their non-professional peers long ago concluded: as a collection and its catalogue grow in size, the usefulness of a subject head-

ing list applied in a dictionary catalogue decreases finally to the point of virtual ineffectiveness". As a remedy that avoids the task of recataloguing a large library, the authors offer this classified list. For the librarian it is supposed to unify the processes of allocating class numbers and subject headings to documents. For the user it is supposed to perform the function of a classified catalogue in providing more effective access to related subjects in a collection. Volume II consists of an alphabetical list of the subject headings to serve as an index to the classified sequence. The main Library of Congress publication, *Subject Headings*, is not considered suitable for this purpose since the subject headings themselves are obscured by a mass of cross-references and instructions.

It is difficult to share the authors' optimism about the value of their work. In the first place it has always been recognized that no published scheme or its index is a suitable guide to the contents of a particular collection. It will inevitably contain many subjects not represented in the library and will fail to list many that are. In the second place, although the authors refer to some shortcomings in the LC system they give no indication that they have taken others into account. They claim, for example, that the classified list eliminates the necessity for the cross-references in the subject headings list. If we were dealing with a first-class classification there would be something in this, but LC is a limited classification. The cross-references in the subject headings list frequently supplement rather than duplicate the relationships shown in the classified order. Furthermore, the correspondence between individual class numbers and headings is far less precise than the presentation implies. This low correlation is dealt with in some detail by J. P. Immroth in his *Analysis of Vocabulary Control in Library of Congress Classification and Subject Headings* (Littleton, Colorado: Libraries Unlimited, 1971).

The idea of applying modern ideas to improve an old system is laudable, but the work under review falls far short of its aim. In fact, the LC system is so faulty that many people would deplore the use of time, money and intellectual effort to prolong its life. They would not like to see repeated in England the recent experience in USA of many large libraries adopting the system—an experience, incidentally, that invalidates the argument that wholesale reorganization of large libraries is unthinkable. No doubt many librarians will continue to defend the use of the system for some purposes, but even they would have to admit that it is least satisfactory in dealing with science. Even with the new publication the scientific research worker cannot hope for any-

thing like an adequate information retrieval service from libraries organized by the LC system. D. W. LANGRIDGE

Ultracentrifugal Topics

Ultracentrifugation of Macromolecules: Modern Topics. By J. W. Williams. Pp. xvi+118. (Academic: London and New York, January 1973.) \$9.50.

IN the year that marks the 50th anniversary of the ultracentrifuge, it is particularly fitting to take notice of the latest contribution from one who was present at the birth. Professor J. W. Williams is justly renowned for his contributions to the theory and use of the ultracentrifuge, an instrument which has played so great a part in our understanding of quantitative relationships among biological macromolecules. Without detracting from the sometimes spectacular advances made by others, it is surely a remarkable achievement on his part to have been consistently in the forefront of innovation and refinement. This volume provides a short account of some areas of special interest to the author, where recent progress has enabled earlier barriers of intractability to be overcome.

The section on the study of synthetic polymers by sedimentation equilibrium shows how the complications arising from polydispersity accompanied by thermodynamic non-ideality can be minimized; it should facilitate the determination of reliable values of molecular weight in these systems.

The field of protein interaction has been one of remarkable development over the past few years, and the sections devoted to this topic provide an excellent illustration of the depths of analysis which Rayleigh optics (usually allied to computer processing of data) have made possible. The determination of equilibrium constants in associating systems also exhibiting non-ideality is indeed impressive when one reflects on how slight are the deviations presented by any one experiment.

A chapter on the determination of size distributions is mostly of historical interest; but the final chapter, on a combination of velocity and equilibrium methods in the study of protein subunits, illustrates the continued value of velocity measurements.

The book concludes with short appendices devoted to the fundamental theory of the ultracentrifuge, and to the effects of molecular heterogeneity. They provide an admirable introduction to the subject, in simply-formulated mathematical terms. If the general approach is that of the physical chemist, the book can nevertheless be studied profitably by all who are concerned with the quantitative interpretation of ultracentrifugal experiments.

J. M. CREETH

CORRESPONDENCE

Smoking, Pregnancy and Publicity

SIR,—Having perused so many methodologically unsound papers concerning associations between smoking and various disorders, I found your leader refreshing and welcome reading. I am relieved to discover that the voice of scientific reason has not been entirely silenced by the well-meaning, if largely misguided, anti-smoking campaign. Nevertheless, I feel your remark "... we cannot, from statistics, infer how or even that smoking is the key to it" needs some qualification.

It is true that we cannot discriminate between, say, causal and constitutional hypotheses by relying exclusively on the usual statistical associations. However, through some ingenious studies, Professor J. Yerushalmy has demonstrated weaknesses and inconsistencies in the causal hypothesis. He found that the perinatal mortality rate and the risk of congenital anomalies were both considerably lower for the low-birth-weight infants of smoking than of non-smoking mothers (*Am. J. Epidemiol.*, **93**, 443; 1971). Furthermore, the best-surviving low-birth-weight infants were born of couples in which the wife smoked and the husband did not: the most vulnerable were produced by couples in which the wife did not smoke and the husband did. These observations show that the connexion between smoking, low birth weight, congenital anomalies and neonatal mortality is complex.

More definite conclusions can be drawn from another of Yerushalmy's studies (*Am. J. Obstet. Gynecol.*, **112**, 277; 1972) in which he explored the question: Are low-birth-weight babies due to the smoking or to the smoker? He found that young mothers who start smoking after the birth of a child have a higher incidence of low-birth-weight infants than those who do not take up the habit. In other words, low-birth-weight infants appear to be attributable to the smoker (or potential smoker), rather than the smoking.

In connexion with the more notorious association between cigarette smoking

and lung cancer, Sir Ronald Fisher (*Br. med. J.*, **2**, 297; 1957) feared that the widely popular causal interpretation would prove to be "... a catastrophic and conspicuous howler". My unanswered letter in the *Lancet* (ii, 102; 1973) provides some further justification for the late Sir Ronald's misgivings.

Yours faithfully,

P. R. J. BURCH

*Department of Medical Physics,
University of Leeds,
The General Infirmary,
Leeds LS1 3EX*

Smoking and Pregnancy

SIR,—I hope I may be allowed a few comments on your editorial¹ which criticises me for claiming 'certainty' for what is only the 'possibility' that maternal smoking causes an increase in perinatal mortality.

You refer to two papers, one in the *British Medical Journal* written with Professor Butler and Dr Ross² and one I wrote for the National Children's Bureau journal *Concern*³. In the former paper we were careful to make the obvious point that the observed statistical association between maternal smoking and perinatal mortality did not of itself imply 'causation' and we suggested that one way of testing the causal hypothesis would be by means of a scientifically controlled health education campaign. The paper in *Concern* was less cautious because it was more concerned with health education, and was written against a background of knowledge that extends beyond the mere statistical associations.

The first piece of knowledge comes from experiments on animals which show that exposure to cigarette smoke during pregnancy does cause a reduction in birth weight and an increase in mortality^{4,5}, and there are good physiological explanations for the mechanisms involved. Second, the medical profession has for a long time been concerned with advising pregnant women (among others) to give up smoking, and this concern was expressed in a recent editorial in the *British Medical Journal*⁶,

which also concluded that the available evidence from human and animal studies suggests that it really is the smoking which causes the association and not, for example, the personality of the women.

Taking up one of your own statements, you say that 'those who can stop smoking during pregnancy should be given every encouragement'. It puzzles me why you should make this statement if you do not really believe that smoking is likely to be harmful. Also, is a health education campaign an 'encouragement' or, as you seem to imply, an undesirable 'social pressure'? I would agree with you that too great a pressure can be counter-productive, and this is a problem that health educators are well aware of. There is undoubtedly much to be learnt about how to warn people of possible dangers, without creating unnecessary alarm or guilt. This does not mean, however, that health education should not be attempted.

When you say that the scientific evidence for a causal relationship may not yet be very conclusive, you are of course correct, and this raises the interesting and important problem of whether and how to use available scientific evidence for purposes of health education. Some research into this might be very useful. In the present instance, however, I retain the opinion that the scientific evidence is strong enough to act upon, and that it would now be unethical not to give advice and encouragement to pregnant women, especially 'high risk' ones, to stop smoking.

Yours faithfully,

HARVEY GOLDSTEIN

*National Children's Bureau,
Adam House,
1 Fitzroy Square,
London W1P 5AH*

¹ *Nature*, **245**, 61 (1973).

² Butler, N. R., Goldstein, H., and Ross, E. M., *Br. med. J.*, **2**, 127 (1972).

³ Goldstein, H., *Concern*, **12**, 26 (1973).

⁴ Younoszai, M. K., Peloso, J., and Haworth, J. C., *Am. J. Obstet. Gynecol.*, **104**, 1207 (1969).

⁵ Schoeneck, W. J., *N.Y. J. Med.*, **41**, 1945 (1941).

⁶ *Br. med. J.*, **1**, 369 (1973).

Obituary

Tom Leadbetter Cottrell

TOM LEADBETTER COTTRELL was born in Edinburgh on June 8, 1923, the son of Allin Cottrell, lecturer in technical chemistry in the University of Edinburgh, and Lily Cottrell, a well known Scottish artist from whom he derived his lifelong interest in art. Cottrell was educated at George Watson's College and at Edinburgh University, where he graduated with first class honours in chemistry in 1943. It is characteristic of the man that while still an undergraduate he worked on agar to gain insight into a line of research he had no intention of pursuing further. In the same year he obtained a position as research chemist with the Nobel Division of ICI and in 1948 was promoted to be head of the physical chemistry research section after a couple of years secondment at Oxford where he gained experience in the Physical Chemistry Laboratory with Dr Leslie Sutton.

Then followed a remarkable period in which Cottrell's interest in the problems he had encountered at Oxford and those engaging his attention at Stevenson resulted in the publication of some thirty papers in ten years. These covered a wide range of topics including the thermal decomposition of nitroalkanes, the heats of combustion of amine nitrates, an electron microscope study of crystal surfaces, as well as theoretical papers in quantum mechanics. These researches were recognized by the award, in 1952, of the Meldola Medal of the Royal Institute of Chemistry. He also found time to write his first book, *The Strengths of Chemical Bonds* (1954), a new edition of which is being prepared

by Dr W. V. Steele of Stirling University.

In 1958 Cottrell became personal assistant to Sir Ewart Smith in the ICI directorate in London, a stimulating and invaluable experience, and a year later he was appointed to the chair of chemistry in Edinburgh University, a rather surprising but imaginative appointment, for Cottrell had little or no experience as a university teacher. He entered into his new duties with characteristic enthusiasm, revised the syllabus in the light of modern developments, established a thriving research school, instituted an honours degree in chemical physics and introduced research on molecular beams under the direction of Dr Fluendy and Dr Lawley. He revived his interest in the spectrophone and, with Arnold Read, successfully overcame the formidable difficulties encountered to obtain results of fundamental importance. In this period he published some twenty papers and three more books, *Molecular Energy Transfer in Gases* (1961), *Dynamic Aspects of Molecular Energy States* (1966), and a small "popular" but stimulating *Chemistry* (1962, second edition 1968), which can be read with profit by novice and expert alike.

Cottrell's six years as professor ended in 1965 when he was appointed Principal and Vice-Chancellor of Stirling University. There can be no doubt that the imaginative and daring qualities he brought to his scientific problems were successfully applied to the foundation of a lively and vigorous institution of learning and research. He took a wide view of the function of a university and the McRobert Centre, the splendid playing fields, and the Outdoor Centre on

Loch Rannoch bear witness to his vision and foresight. Only in time will his achievements as first Principal be fully recognized.

The demands of administration did not prevent Cottrell maintaining his interest in research especially on electron impact spectroscopy, begun in Edinburgh and continued by Dr Isobel Walker, one of his former Edinburgh students and the first lecturer in chemistry to be appointed at Stirling. Just before he died he hoped to obtain sabbatical leave in 1973-74 and return for a spell of active research. This was not to be. The university has lost a brilliant and zestful leader and chemistry a bold pioneer who had the gift of spotting important and exciting problems and who tackled them no matter how difficult and daunting they might be. His main theme was the energy involved in chemical and physical processes and in particular the rate at which energy transfer occurs. He was unusually well equipped to deal with the theoretical aspects of his problems and he employed ingenious experimental techniques. The heavy calls on his time and his untimely death prevented him fulfilling himself as a scientist; but the wonder is that he accomplished so much.

Tom Cottrell was a buoyant personality with an engaging modesty, a keen sense of humour and an interest in literature, art and music. He wore his learning lightly, found relaxation in yachting and was strengthened in difficult times by a devoted wife. A crowded congregation at the memorial service in the Church of the Holy Rude in Stirling testified to the respect in which he was held.

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